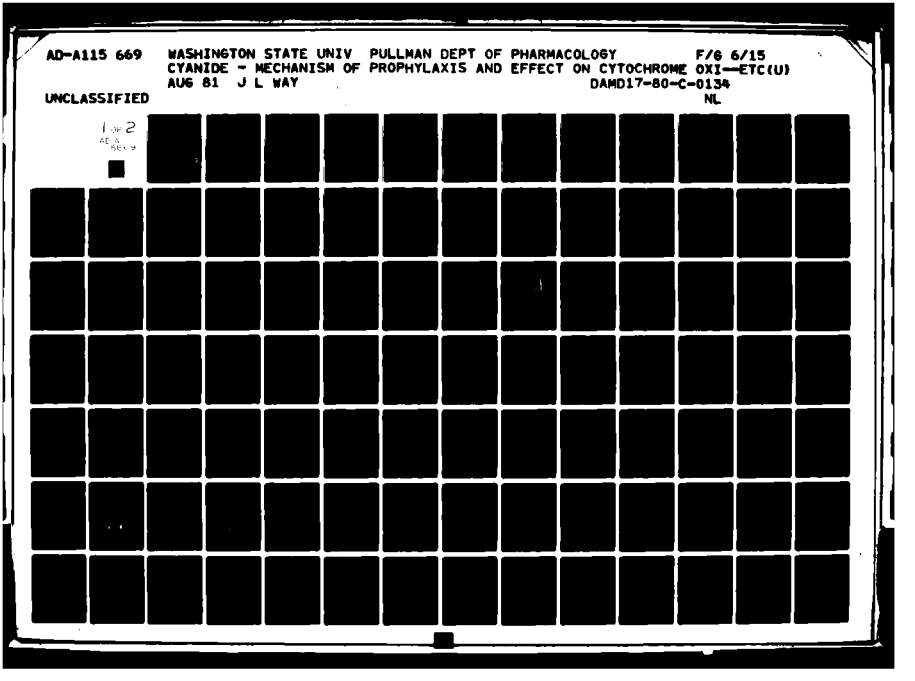
AD-A115 669 WASHINGTON STATE UNIV PULLMAN DEPT OF PHARMACOLOGY F/G 6/15
CYANIDE - MECHANISM OF PROPHYLAXIS AND EFFECT ON CYTOCHROME OXI--ETC(U)
AUG 81 J L WAY DAMD17-80-C-0134
UNCLASSIFIED NL



AD _____

CYANIDE - MECHANISM OF PROPHYLAXIS AND EFFECT ON CYTOCHROME OXIDASE

Annual Report

James L. Way, Principle Investigator

August 15 1981

Supported by

U. S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
Fort Detrick, Fredrick, Maryland 21701

Contract No. DAMD17-80-C-0134

Department of Pharmacology
Washington State University
Pullman, Washington 99164

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

REPORT DOCUMENTATION PAGE		READ INSTRUCTIONS BEFORE COMPLETING FORM
1. REPORT NUMBER	2. GOVT ACCESSION NO.	3. RECIPIENT'S CATALOG NUMBER
4. TITLE (and Subtitle) CYANIDE - MECHANISM OF PROPHYLAXIS AND EFFECT ON CYTOCHROME OXIDASE		5. TYPE OF REPORT & PERIOD COVERED Annual Report Sept 15 1980 - Aug 15 1981 6. PERFORMING ORG. REPORT NUMBER
7. AUTHOR(s) James L. Way		8. CONTRACT OR GRANT NUMBER(s) DAMD 17-80-C-0134
9. PERFORMING ORGANIZATION NAME AND ADDRESS Department of Pharmacology Washington State University Pullman, Wa 99164		10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS
11. CONTROLLING OFFICE NAME AND ADDRESS U. S. Army Medical Research and Development Command Fort Detrick, Fredrick, ML 21701		12. REPORT DATE 8/15/81 13. NUMBER OF PAGES 109
14. MONITORING AGENCY NAME & ADDRESS(if different from Controlling Office)		15. SECURITY CLASS. (of this report) Unclassified 15a. DECLASSIFICATION/DOWNGRADING SCHEDULE

16. DISTRIBUTION STATEMENT (of this Report)

Statement A: Approved for public release; distribution unlimited

17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)

18. SUPPLEMENTARY NOTES

19. KEY WORDS (Continue on reverse side if necessary and identify by block number)

Cyanide, Chlorpromazine, Cytochrome oxidase

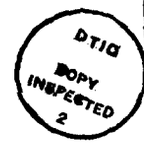
20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

see SUMMARY section of report

DD FORM 1 JAN 73 1473 EDITION OF 1 NOV 65 IS OBSOLETE

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

CYANIDE - MECHANISM OF PROPHYLAXIS AND EFFECT ON CYTOCHROME OXIDASE



SUMMARY

ABSTRACT

Way, James L., Alan Kong, Diane Sylvester, Gary E. Isom and George E. Burrows: Effect of Chlorpromazine on Cyanide Intoxication. J. Pharmacol. Exp. Ther.

Previous reports from our laboratory indicated that the prophylactic protection against cyanide intoxication in mice can be enhanced by the administration of chlorpromazine when it is given with sodium thiosulfate either alone or with sodium nitrite. The mechanism of the potentiation of sodium thiosulfate by chlorpromazine was studied alone and in various combinations with the classic cyanide antidotal combination of sodium nitrite and sodium thiosulfate. Although chlorpromazine was found to induce a hypothermic response, these studies indicate that the mechanism of enhancement of the antagonism of cyanide by chlorpromazine does not correlate with the hypothermia produced. Various other possible mechanisms were investigated, such as, rate of methemoglobin formation, enzymatic activity of brain and liver rhodanese and cytochrome oxidase, α adrenergic blocking properties, and the overall effect on glucose oxidation as elicited by radiorespirometric studies. Although the pharmacologic effects of chlorpromazine are complex the α-adrenergic blocking properties of chlorpromazine would provide a basis for its antidotal effect against cyanide intoxication.

George E. Burrows, David H. W. Liu and James L. Way. Effect of Antagonists on the Physiological Disposition of Cyanide. Toxicol. Appl. Pharmacol.

Attempts were made to evaluate the effects of air and oxygen either alone or in various combinations with sodium nitrite and/or sodium thiosulfate on the physiological disposition of carbon-14 sodium cyanide in mice. The radioactive respiratory gases were studied by radiorespirometry, and the effects of various combinations of cyanide antagonists were compared. Oxygen either alone or in combination with sodium thiosulfate significantly enhanced the respiratory excretion as compared with air. Sodium thiosulfate accelerated the initial rate of excretion of radioactivity in both air and oxygen-treated animals. Sodium thiosulfate accelerated the initial rate, but not the total amount of radioactivity excreted, whereas oxygen increased the amount of radioactivity excreted, but did not affect the initial rate of excretion. The cumulative recovery of radioactive gases was consistently greater in the groups given oxygen over the respective groups given air, but the difference was significant only with groups receiving oxygen alone or with sodium thiosulfate. When sodium nitrite was employed as an antidote either alone or with sodium thiosulfate, no difference in the respiratory excretion was noted between air and oxygen. The use of the sodium nitrite-sodium thiosulfate combination either with air or oxygen resulted in a marked decrease in the initial rate as well as total amount of radioactivity excreted.

ABSTRACT

ISOM, GARY E., AND WAY, JAMES L.: Alteration of the biochemical effects of cyanide with oxygen.

The inhibition and recovery of brain and liver cytochrome oxidase in mice pretreated in an air or oxygen atmosphere were measured after the administration of KCN with and without sodium nitrite and sodium thiosulfate pretreatment. Inhibition of cytochrome oxidase in both brain and liver reached a maximum within five minutes after cyanide administration, and cytochrome oxidase activity was restored more rapidly in liver than in brain. This enzymatic activity returned more rapidly in oxygen than air. In the animals pretreated with sodium nitrite and sodium thiosulfate, brain but not liver cytochrome oxidase was inhibited by cyanide. The effect of administering gradient doses of KCN to mice maintained in air or oxygen atmospheres resulted in a dose dependent inhibition of both brain and liver cytochrome oxidase. Oxygen treatment produced a shift to the right in the dose response curve when compared to the air treatment group. No significant difference was detected in rhodanese activity in air and oxygen in mice receiving a gradient dose of cyanide.

In conducting the research described on this report, the investigators adhered to the "Guide for Laboratory Animal Facilities and Care", as promulgated by the Committee on the Guide for Laboratory Animal Resources, National Academy of Sciences- National Research Council

TABLE OF CONTENTS

Appendixes, illustrations, and tables are included in the body of each individual section.

SECTION 1

Effect of Chlorpromazine on Cyanide Intoxication

Chlorpromazine has been reported to antagonize the lethality of cyanide in pigeons (Guth and Spirtes, 1958). Subsequently, this antidotal effect was investigated in mice, and the mechanism was attributed predominantly to the hypothermia induced by chlorpromazine (Levine and Klein, 1959).

Chlorpromazine has been reported to produce a fall in body temperature in many species including man (Ayd, 1955), dog (L'Allemand et al., 1955), guinea pig (Chevillard et al., 1958), rabbit (Bachtold and Pletscher, 1957), mouse (Kopera and Armitage, 1958), rat (Kirkpatrick and Lomax, 1971), hamster and ground squirrel, but not pigeon (Hoffman and Zarrow, 1958). Yet, surprisingly, the initial observation of chlorpromazine exerting a protective effect against cyanide intoxication was reported in pigeon. Also, it has been reported in in vitro studies that chlorpromazine is an inhibitor of the cyanide sensitive enzyme, cytochrome oxidase (Bernsohn et al., 1956). This latter observation is of interest since one would anticipate an enhancement rather than an antagonism of cyanide intoxication by chlorpromazine, as cyanide lethality has been attributed to a decrease in tissue utilization of oxygen, presumably by inhibition of cytochrome oxidase (Keilin, 1930; Warburg, 1931; Albaum et al., 1946; Schubert and Brill, 1968).

The above paradoxical situations warrant further investigation concerning the mechanism of antagonism of cyanide intoxication by chlorpromazine, particularly when it is employed in combination with the classic cyanide antagonists, sodium nitrite and sodium thiosulfate (Chen et al., 1933). These current studies were initiated in an attempt to investigate the mechanism of the antidotal properties of chlorpromazine on various biochemical and physiological parameters.

MATERIALS

Potassium cyanide was purchased from Fischer Scientific Company (Fairlawn, NJ). Sodium nitrite, sodium thiosulfate, potassium phosphate, ferric nitrate, potassium thiocyanate, and 38% formaldehyde were products of the J. T. Baker Chemical Company (Phillipsburg, NJ). Chlorpromazine was obtained from Smith, Kline and French Laboratories (Philadelphia, PA), and Tris was obtained from Calbiochem (La Jolla, CA). Glucose and nitric acid were purchased from the Mallinckrodt Chemical Works (St. Louis, MO), and uniformly labeled ^{14}C-glucose (glucose-U-^{14}C) and Aquasol was obtained from New England Nuclear Corporation (Boston, MA). Compressed air was a product of the Industrial Air Products (Portland, OR). All other chemicals employed in these studies were of the highest purity commercially available.

METHODS

Male Swiss Webster mice weighing between 18-24 gms, were housed in air-conditioned rooms maintained at 20-23°C. The mice were divided randomly into various groups of four or more mice per group. Dose and route of administration were as follows: Sodium nitrite (100 mg/kg, i.p.), chlorpromazine (10 mg/kg, i.p.), methoxamine (10 mg/kg, i.p.), and sodium thiosulfate (1 g/kg, i.p.) were administered 45, 30, 20, and 10 minutes respectively prior to the injection of potassium cyanide (5-45 mg/kg, s.c.). All mice were sacrificed by decapitation. Blood samples were obtained after decapitation and methemoglobin was determined by the method of Leahy and Smith (1960). The rectal temperature was measured with a thermister probe and a telethermometer (Yellow Spring Instrument Co., Yellow Spring, OH). Radiorespirometric studies with glucose-U-^{14}C were conducted as previously described (Isom and Way, 1974). The area under the radiorespirometric curve was determined with a planometer and correlated by cutting out the

area under each curve and weighing it. One mouse is used in each radiorespirometric study and the mean of four or more curves are determined in each experiment, using a different mouse for each radiorespirometric study.

Cytochrome Oxidase Assay

At specific time intervals after administration of cyanide and its antagonists, the mice were sacrificed by decapitation, the brain was excised quickly, and excess blood was removed by rinsing in cold saline. A 2% (w/v) crude homogenate was prepared in chilled distilled water (2°C) and diluted 10 fold with 0.02 M tris-HCl buffer, pH 7.4. The incubation mixture in the spectrophotometer cuvette contained one-half ml of cytochrome c solution (10^{-4} M, reduced with sodium dithionate), and 2.4 ml of 0.03 M tris-HCl buffer. The density of 0.1 ml of the 0.2% (w/v) brain homogenate was determined at 550 nm in a Beckman model *DU spectrophotometer*. Finally 0.05 ml of saturated potassium ferricyanide solution was added to completely oxidize the cytochrome c and the optical density was redetermined. Cytochrome oxidase activity was expressed as the first order rate constant (Cooperstein and Lazarow, 1951).

Protein concentration of the 0.2% (w/v) brain homogenate was determined by the method of Lowry (1951), and the rate constant was expressed in terms of milligrams of protein per unit time (mg^{-1} sec^{-1}). The enzymatic activity of the brain homogenates were determined in duplicate and 4 or more animals were used per treatment group.

Rhodanese Assay

The method of Sörbo (1955) was modified as follows: After the specific drug treatments, brain tissue was removed from the animal as rapidly as possible. A 5% (w/v) brain homogenate was prepared with 0.0125 M sodium thiosulfate. One-half ml of the homogenate was added to an incubation mixture containing 1 ml

$Na_2S_2O_3$ (0.125 M), 0.5 ml KH_2PO_4 (0.20 M) and 0.5 ml KCN (0.25 M) and shaken in a water bath for 5 minutes at 37.5°C in stoppered 25 ml erlenmeyer flasks. The reaction was stopped with 0.5 ml of 38% formaldehyde solution. Then 2.5 ml of ferric nitrate reagent was added and subsequently diluted with 20 milliliters of distilled water and centrifuged at 6,000 x g for 10 minutes. The absorbance was recorded at 460 nm with water in the reference cuvette, and the enzymatic activity was expressed as mg of cyanide converted to thiocyanate per gram of tissue. Standard curves were determined by adding known amounts of potassium thiocyanate to the incubation mixture containing formaldehyde.

Potency Ratio Determination

Mice were distributed randomly into experimental groups, and the effects of various treatments on the lethality of cyanide were assessed. The LD_{50} values, which were based on 24-hour mortality, were determined in five or more groups that contained ten mice each. Respective slopes of the dose-response curves for each experiment employing a cyanide antagonist, or antagonists, were found to be not significantly different from that for cyanide alone. The LD_{50} values of these experiments were statistically analyzed according to the method of Litchfield and Wilcoxon (1949). The confidence limits of the LD_{50} values are expressed as 19/20 probability. After plotting the regression lines and determining that the slopes of log dose-mortality curves were not significantly different from each other, the LD_{50} values of potassium cyanide with and without antagonist(s) were compared by means of the potency ratios. The potency ratios were then statistically compared.

Statistical Analyses

Standard errors and unpaired student's test were computed on a Wang 600 programmable calculator (Wang Laboratories, Tewksburg, MA). A probability level

of 0.05 was used in testing for signifant differences between experimental groups.

RESULTS

Investigation of the mechanism of chlorpromazine's antagonism of cyanide lethality was a result of our earlier toxicity studies (Way and Burrows, 1976) showing that only when chlorpromazine is combined with sodium thiosulfate, either alone or with sodium nitrite, that a potentiating effect is observed.

Concentrations of methemoglobinemia are measured and evaluated with the rates of formation after the administration of chlorpromazine, sodium nitrite, sodium thiosulfate, and a combination of sodium nitrite and chlorpromazine. The rate of formation of methemoglobin is quite rapid and maximal development of 30 to 40% methemoglobin concentration occurs between 30 to 45 minutes (Figure 1). Chlorpromazine either alone or in combination with sodium thiosulfate did not enhance the formation of methemoglobin. Also, the combination of chlorpromazine with sodium nitrite did not enhance the rate of formation nor the amount of methemoglobin formed.

In order to evaluate the hypothermic response induced by chlorpromazine in these experiments, it was necessary to investigate the effect of cyanide under various conditions without chlorpromazine (Figure 2a). A transient fall in body temperature was elicited in most experimental groups with a maximal hypothermic response occurring approximately 30 minutes after cyanide administration except when sodium nitrite was employed. When sodium nitrite is administered with potassium cyanide, the induced hypothermia is maximal at time zero and the body temperature is rising in spite of the administration of potassium cyanide. The severity of the hypothermia produced appears to correlate to some extent with the dose of potassium cyanide, as potassium cyanide alone (5 mg/kg) produced the least hypothermic response, whereas the sodium nitrite-sodium thiosulfate combination with potassium cyanide (45 mg/kg) produced the most severe

hypothermia. When chlorpromazine is included in these studies (Figure 2b), the hypothermia produced was not only enhanced in intensity, but sustained over a longer duration of action. The intensity of the hypothermia produced did not correlate with the dose of potassium cyanide as in the studies without chlorpromazine. In fact, chlorpromazine alone produced the greatest fall in body temperature, and any combination of chlorpromazine with potassium cyanide and/or its antagonists lessened the hypothermic response. When chlorpromazine is administered alone, a maximal hypothermia occurs at 90 to 120 minutes (Figure 2b); however, when chlorpromazine is administered with cyanide, an antagonism in the hypothermia occurs and the rise in body temperature is sustained for 60 minutes before a decrease in temperature is resumed. In all experiments the hypothermia induction was more greatly enhanced with chlorpromazine. It is of interest to note that the most rapid onset in hypothermia was elicited with the combination of sodium nitrite and chlorpromazine.

The effect of chlorpromazine on cytochrome oxidase was investigated, as it was reported to be inhibitory in in vitro studies (Bernsohn et al, 1956). An in vivo stimulation of cytochrome oxidase activity by chlorpromazine (Dodson et al, 1975) could not be confirmed. The effect of chlorpromazine on various pretreatments in mice, as previously described, was examined with respect to brain cytochrome oxidase (Figure 3). Animals receiving potassium cyanide (5 mg/kg) elicited a 62% inhibition of brain cytochrome oxidase, and the addition of chlorpromazine had no effect on this cyanide induced inhibition. Animals receiving sodium nitrite and potassium cyanide (10 mg/kg) resulted in a 45% inhibition of cytochrome oxidase and the combination of sodium nitrite with chlorpromazine resulted in a further decreased inhibition of cytochrome oxidase. Sodium thiosulfate has no apparent effect on cytochrome oxidase and when it was administered with chlorpromazine a decrease in the

percent of cytochrome oxidase inhibition was manifested. The classic cyanide antidotal combination with potassium cyanide produced a 43% inhibition and the addition of chlorpromazine to this combination had no significant effect. It should be pointed out that only each paired experiment can be compared and the cross comparison between paired groups is not valid, since the dose of potassium cyanide varied with each of the antidotal combinations.

The effect of chlorpromazine on rhodanese activity in the liver and brain homogenates was investigated and the various treatment groups were found to have no significant effect on brain rhodanese activity. The in vivo inhibitory effect of potassium cyanide and/or sodium nitrite either alone or in combination with chlorpromazine on liver rhodanese could not be confirmed (Dodson et al., 1975), as the rhodanese activity was found to be no different with or without cyanide or nitrite.

The inhibitory action of chlorpromazine either alone or in various combinations with sodium nitrite, sodium thiosulfate and potassium cyanide on acute glucose oxidation was investigated by radiorespirometry by determining the peak interval (Figure 4) and cumulative yield. In animals receiving glucose-U-^{14}C and treated with saline (control) a peak respiratory excretion of 12.5% of the administered reactivity as $^{14}CO_2$ in a 6-hour period. Potassium cyanide caused a shift in the maximal excretion of $^{14}CO_2$, and the peak respiratory excretion was displaced from 60 to 180 minutes. Approximately 53% of the administered glucose-U-^{14}C was excreted as carbon dioxide. The above results are consistent with our earlier studies (Isom and Way, 1974). When chlorpromazine alone was administered without KCN, a sharp decrease in the oxidation of glucose-U-^{14}C was noted (Figure 4). The peak interval yield was decreased to 1%, and the cumulative yield was depressed to 12%. A possible shift in the peak excretion

of $^{14}CO_2$ was not apparent, as the peak interval curve was too greatly depressed. Similar radiorespirometric data was obtained when chlorpromazine was administered in combination with potassium cyanide and/or with sodium nitrite. It is of interest to note that with respect to the chlorpromazine-sodium thiosulfate combination, the inhibitory effect of chlorpromazine was significantly reversed (Figure 4). In the mice receiving the chlorpromazine-sodium thiosulfate combination, the peak respiratory excretion of glucose-U-^{14}C as CO_2 was increased to 3.5% of the radioactivity administered, and the radiorespirometric excretion occurred at 90 minutes (Figure 4). The cumulative yield of CO_2 derived from glucose-U-^{14}C after six hours was approximately 32%. It is apparent that the sodium thiosulfate-chlorpromazine combination enhances the oxidation of glucose when compared to the combination of KCN-chlorpromazine.

Since chlorpromazine is believed to possess α adrenergic blocking properties, attempts were made to study the effect of an α agonist, e.g., methoxamine,on the protective effect of chlorpromazine in cyanide poisoning (Figure 5). The antidotal potency ratios, expressed as the LD_{50} of antagonized cyanide over unantagonized cyanide, illustrates the overall beneficial effect of chlorpromazine under certain conditions to protect against cyanide poisoning, as we have reported earlier (Way and Burrows, 1976). However, this chlorpromazine potentiation against the lethal effects of cyanide was reversed by methoxamine. It should be pointed out that all possible combinations of the LD_{50} values of all experimental groups were compared statistically by means of the potency ratio so that the cross comparison of any group would be valid. Under these circumstances, it is apparent that chlorpromazine either alone or in combination with sodium nitrite does not enhance the protection against the lethal effects of potassium cyanide (Way and Burrows,

1976), and methoxamine has no apparent effect on either of these two antidotal combinations. When chlorpromazine was employed with sodium thiosulfate, either alone or in combination with sodium nitrite, the potency ratios were enhanced. However, the addition of methoxamine to the above antidotal combinations appeared to antagonize the synergistic effect of chlorpromazine, as the potency ratios of the methoxamine-treated animals were no different from those animals which received no chlorpromazine.

DISCUSSION

The interpretation of these results is complex, since so many drugs were employed in this experimental design. Also these studies are attempting to relate the pharmacologic effect of very high, but still sublethal doses of cyanide, with the mechanisms of lethality. The basis of these studies are whether chlorpromazine induced hypothermia, as reported by other laboratories, contributes significantly to the antagonism of cyanide antagonism, and whether some alternative pharmacologic properties of chlorpromazine may provide a basis for this antidotal effect.

Under the conditions of these studies, it seems that the chlorpromazine induced hypothermia probably bears very little, if any, relationship to the antagonism of cyanide intoxication. Chlorpromazine produces the greatest decrease in body temperature without sodium thiosulfate; yet chlorpromazine alone provides no protective effect, whereas with sodium thiosulfate a striking potentiation of the protective effect is noted. Also, one of the most rapid falls in body temperature and most severe hypothermia is produced by the sodium nitrite-chlorpromazine combination, and chlorpromazine again does not increase the protective effect of sodium nitrite. Lastly, the smallest net decrease in body temperature with chlorpromazine is noted with the sodium nitrite-sodium thiosulfate combination; yet, a significant increased protective effect is noted when chlorpromazine is added. The hypothesis that chlorpromazine induces hypothermia, causes a decrease in body requirement for oxygen and thereby protects against cyanide toxicity is a reasonable one: however, the present studies indicate there appears to be no relationship between the protective effect of chlorpromazine with its drug-induced hypothermia.

With respect to the effects of chlorpromazine on cytochrome oxidase, brain preparations were employed, as it is presumed that the predominant site of cyanide lethality is central. Although considerable changes in cytochrome oxidase activity were noted in these studies, there appears to be no consistent relationship between the protective effect of chlorpromazine with brain cytochrome oxidase activity. It should be pointed out that chlorpromazine in combination with sodium nitrite does not enhance the protective effect of sodium nitrite against cyanide lethality. These results are of interest, as this combination produced one of the greatest hypothermic response and the least inhibition of cytochrome oxidase. With a decreased demand on oxidative processes due to the induced hypothermia, and decreased inhibition of the terminal oxidative enzymes presumably through competition with methemoglobin for cyanide, one would have expected this combination to have exerted some enhanced protective effect against the lethality of cyanide intoxication. It is not surprising that sodium thiosulfate has little effect on cytochrome oxidase activity, as the distribution of this sulfur donor to the central nervous system is limited.

The enzymatic activity of rhodanese in brain and liver to detoxify cyanide was not affected by chlorpromazine. Although chlorpromazine contains a sulfur atom and may serve as a substrate for rhodanese, the substrate specificity of this enzyme has been investigated by Sörbo (1953a,b), and chlorpromazine should not serve as a sulfur donor for this enzyme.

Studies on glucose oxidation by radiorespirometric studies were prompted primarily because our earlier studies with oxygen indicated that the antagonism of cyanide toxicity also resulted in a reversal of cyanide inhibition of glucose

oxidation (Isom and Way, 1974). It was of interest to determine if chlorpromazine exerted a similar biochemical effect. Stimulation of anaerobic glycolysis by cyanide is attributed to the inhibition of cytochrome oxidase resulting in a shift from aerobic to anaerobic metabolism (Albaum et al., 1946; Estler, 1965; Detwiler, 1972; Isom and Way, 1974). Unfortunately chlorpromazine alone has a profound effect on glucose oxidation. This striking decrease in glucose oxidation may be partly but not totally related to the induced hypothermia (Fisher et al., 1958; Maur et al., 1962; Fuhrman and Fuhrman, 1963; Hennerman et al., 1958). The correlation of the reversal of cyanide toxicity and glucose oxidation, as had occurred in the presence of oxygen (Isom and Way, 1974) would probably be masked by the inhibitory effect of chlorpromazine itself on glucose oxidation. It is of interest to note that sodium thiosulfate alone inhibits glucose oxidation (Isom and Way, 1974); however, when it is combined with potassium cyanide and chlorpromazine, all of which are known to inhibit glucose oxidation, the net result is an enhanced glucose oxidation over that of chlorpromazine either alone or with potassium cyanide.

Since chlorpromazine does possess α adrenergic blocking properties, attempts were made to relate this pharmacologic action of chlorpromazine with its ability to enhance the antagonism of cyanide lethality. Subsequent to these studies, other α adrenergic blocking agents such as phenoxybenzamine have been found to antagonize the lethal effect of cyanide (Burrows et al., 1977). The pattern of the cyanide antagonism produced by these α adrenergic blocking agents resemble those of chlorpromazine, i.e., they produce no enhancement of the antagonism of the lethal effects of cyanide either alone or in combination with sodium nitrite, and they strikingly potentiate the effects

of sodium thiosulfate and enhance the protective effect of the sodium nitrite-sodium thiosulfate combination.

The ability of methoxamine to reverse the protective effect of chlorpromazine provides a possible mechanism for its protective action. This is of particular interest, as when chlorpromazine does not enhance the protective effect against cyanide lethality, methoxamine appears to have no effect. As the dose of cyanide is increased, various other toxic mechanism may come into play other than the inhibition of cytochrome oxidase. For example, autonomic shock from the release of biogenic amines may play a role in the lethal effects of high doses of cyanide and chlorpromazine may be exerting a protective effect through α adrenergic blockade. A more detailed investigation of the role of α adrenergic blockade in cyanide lethality is projected with phenoxybenzamine rather than with chlorpromazine, because of the relative ease of pharmacologic analysis of the former compound.

In conclusion, various mechanisms were investigated on how chlorpromazine antagonizes the lethal effects of cyanide intoxication. This protective effect clearly is not related to the hypothermic response as proposed by other investigators. The α adrenergic blocking property of chlorpromazine provides a basis for this antidotal effect of chlorpromazine.

REFERENCES

Albaum, H. G., Tepperman, J., and Bodansky, O.: The in vivo inactivation by cyanide on brain cytochrome oxidase and its effect on glycolysis and on the high energy phosphorous compounds in brain. J. biol. Chem. 164:45-51 1946.

Ayd, F. J.: The physiologic and neurologic action of chlorpromazine. Psychiat. Res. Rep. 1:14-44, 1955.

Bachtold, H. and Pletscher, A.: Einfluss von isonikotinsaurehydraziden auf den verlauf der korper temperatur nach reserpine, monoaminen and chlorpromazine. Experientia 13:163-165, 1957.

Bernsohn, J., Namajuska I., and Boshes, B.: The action of chlorpromazine and reserpine on brain enzyme systems. J. Neurochem. 1:145-149, 1956.

Burrows, G. E., King, L., Tarr, S., and Way, J. L.: The protective effect of vasoactive compounds as cyanide antagonist. Proc. Int. Cong. on Toxicol. 48, 1977.

Chen, K. K., Rose C. L. and Clowes, G. H. A.: Methylene blue, nitrites and sodium thiosulfate against cyanide poisoning. Proc. Soc. exp. Biol. Med. 31:250-252, 1933.

Chevillard, L., Giorno, H. and Lauri, M.: Action comparee de quelques vasodilateurs sur la temperature corporelle et la metabolisme respiratoira chez le cobayle t.r. Seanc. Soc. Biol. 152:1074-1077, 1958.

Cooperstein, S. J. and Lazaro, A.: A microspectrophotometric method for the determination of cytochrome oxidase. J. biol. Chem. 189:665-670, 1951.

Detwiler, T. L.: Control of energy metabolism in platelets. The effects of thrombin and cyanide on glycolysis. Biochem. biophys, Acta. 256:163-174, 1972.

Dodson, R. A., Burrows, G. E., Isom, G. E., and Way, J. L.: Mechanism of chlorpromazine antagonism of cyanide intoxication. Proc. West. Pharm. Soc. 18:348-350, 1975.

Estler, C. L.: Stoffwechselveranderungern des Gehirns im Verlauf der nicht lethalen Kaliumcyanidvergiftung und ihre Beeinflussung durch Cyanidantagonisten. Nauny-Schmiedebergs Arch. Pharmakol. 251:413-442, 1965.

Fisher, B., Fedor, E. J., and Lec. S. H.: Rewarming following hypothermia of two to twelve hours. II. Some metabolic effects. Ann. Surg. 148:32-42, 1958.

Fuhrman, G. J., and Fuhrman, F. A.: Utilization of glucose by the hypothermic rat. Amer. J. Physiol. 205:181-183, 1963.

Guth, P. S. amd Spirtes, M. A.: Antagonism of cyanide intoxication by chlorpromazine. Fed. Proc. 17:374, 1958.

Hennerman, D. H., Bunker, J. P., and Brewster, J. W. R.: Immediate metabolic response to hypothermia in man. J. Appl. Physiol. 12:164-168, 1958.

Hoffman, R. A. and Zarrow, M. T.: Hypothermia in the rat, hamster, ground squirrel and pigeon following chlorpromazine. Amer. J. Physiol. 193:547-552, 1958.

Isom, G. E., and Way, J. L.: Effect of oxygen on cyanide intoxication. IV. Reactivation of cyanide-inhibited glucose metabolism. J. Pharmacol. Exp. Ther. 189:235-243, 1974.

Keilin, D.: Cytochrome in intracellular oxidase. Proc. R. Soc. 106:418-444, 1930.

Kirkpatrick, W. E. and Lomax, P.: Temperature changes induced by chlorpromazine and N-methyl chlorpromazine in the rat. Neuropharmacology 10:61-66, 1971.

Kopera, J. and Armitage, A. K.: Comparison of some pharmacological properties of chlorpromazine, promethazine and pethidine. Brit. J. Pharm. Chemother. 9: 392-401, 1958.

L'Allemand, H. J., Brendel, W. and Unsing, W.: Uber den mechanisms der chlorpromazine-(megaphen)-wirkung auf die temperatur regulation. Der Anesthesist. 41:36-41, 1955.

Leahy, T., and Smith, R.: Notes on methamoglobin determinations. Clin. Chem. 6: 148-152, 1960.

Levine, S. and Klein, M.: Effect of chlorpromazine on cyanide intoxication. Proc. Soc. Exp. Biol. Med. 102:192-194, 1959.

Litchfield, J. T., Jr. and Wilcoxon, F.: A simplified method of evaluating dose-effect experiments. J. Pharmacol. Exp. Therap. 96:99-113, 1949.

Lowry, C. H., Roschbrough, N. J., Farr, A. L., and Randall, R. J.: Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193:265-275, 1951.

Maur, J. M., McComiskey, D. M., Kaunes, J. W., and Beaton, J. R.: Carbohydrate metabolism in hypothermic rats and hamsters. Canad. J. Biochem. Physiol. 40: 1427-1438, 1962.

Schubert, J. and Brill, W. A.: Antagonism of experimental cyanide toxicity in relation to the in vivo activity of cytochrome oxidase. J. Pharmacol. Exp. Ther. 162:352-359, 1968.

Sörbo, B. H.: Crystalline rhodanese. I. Purification and physiocochemical examination, Acta Chem. Scand. 7:1129-1136, 1953a.

Sörbo, B. H.: On the substrate specificity of Rhodanese. Acta Chem. Scand. 7:32-37, 1953b.

Sörbo, B. O.: Rhodanese. In Methods in Enzymology, ed. by S. P. Colowick and N. O. Kaplan, 2:334-337, Academic Press, Inc., New York, 1955.

Warburg, O.: Uber nicht-hemmung der zellatmung durch blausaure. Biochem. Z. 231:493-497, 1931.

Way, J. L., and Burrows, G.: Cyanide intoxication: Protection with chlorpromazine. Tox. and Appl. Pharm. 36:93-97, 1976.

Fig. 1. Rate and total formation of methemoglobin in mice after $NaNO_2$ (100 mg/kg), $Na_2S_2O_3$ (1 gm/kg) and chlorpromazine (10 mg/kg)administration. Methemoglobin is expressed as percentage of total hemoglobin. ▲—▲ $NaNO_2$; ●---● $NaNO_2$ + CPZ; □---□ CPZ; ○—○ $Na_2S_2O_3$.

Fig. 2a. The effect of KCN and antagonists without chlorpromazine on the mean rectal temperature in mice. ○---○ Saline control; ●—● KCN (5 mg/kg); Δ—Δ $NaNO_2$ (100 mg/kg) + KCN (10 mg/kg); ○—○ $Na_2S_2O_3$ (1gm/kg) + KCN (15 mg/kg); Δ---Δ $NaNO_2$ (100 mg/kg) + $Na_2S_2O_3$ (1gm/kg) + KCN (45 mg/kg).

Fig. 2b. The effect of KCN and antagonists in combination with chlorpromazine (10 mg/kg) on the mean rectal temperature in mice. ○—○ Saline control; ●—● CPZ; Δ—Δ CPZ + KCN (5 mg/kg); ▲---▲ CPZ + $NaNO_2$ (100 mg/kg) + KCN (10 mg/kg); ○---○ CPZ + $Na_2S_2O_3$ (1 gm/kg) + KCN (15 mg/kg); Δ---Δ CPZ + $NaNO_2$ (100 mg/kg) + $Na_2S_2O_3$ (1gm/kg) + KCN (45 mg/kg).

Fig. 3. Percent inhibition of mouse brain cytochrome oxidase by KCN <u>in vivo</u> with and without antagonists.

Fig. 4. Respiratory excretion of $^{14}CO_2$ at 30 minute intervals from mice administered glucose-U-^{14}C after pretreatment with various combinations of KCN (5 mg/kg), chlorpromazine (10 mg/kg), $NaNO_2$ (100 mg/kg) and $Na_2S_2O_3$ (1gm/kg). ● —— ● Saline control; ▲ —— ▲ KCN; ● —— ● CPZ + $Na_2S_2O_3$ + KCN; ○ —— ○ CPZ + $NaNO_2$ + KCN; ■ --- ■ CPZ; △ --- △ KCN + CPZ.

Fig. 5. Antidotal potency ratios of KCN with and without antagonist. In KCN (control), isotonic saline was employed as the antagonist. Potency ratio = KCN with antagonist(s) /KCN without antagonist(s).

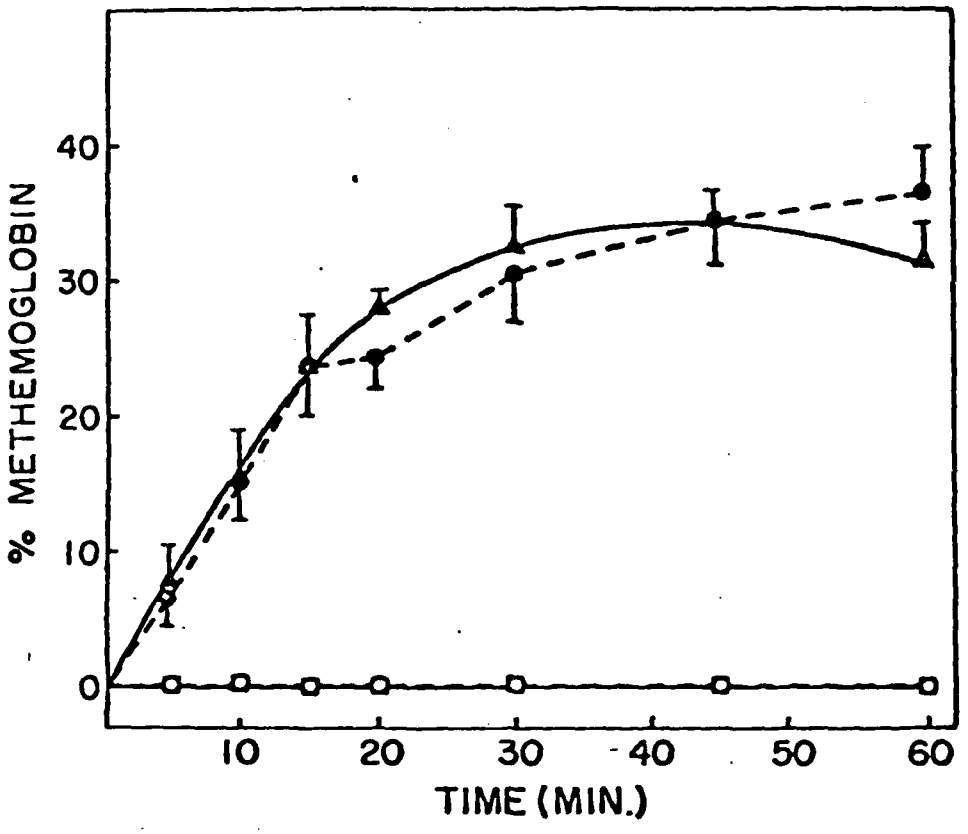


Fig 1

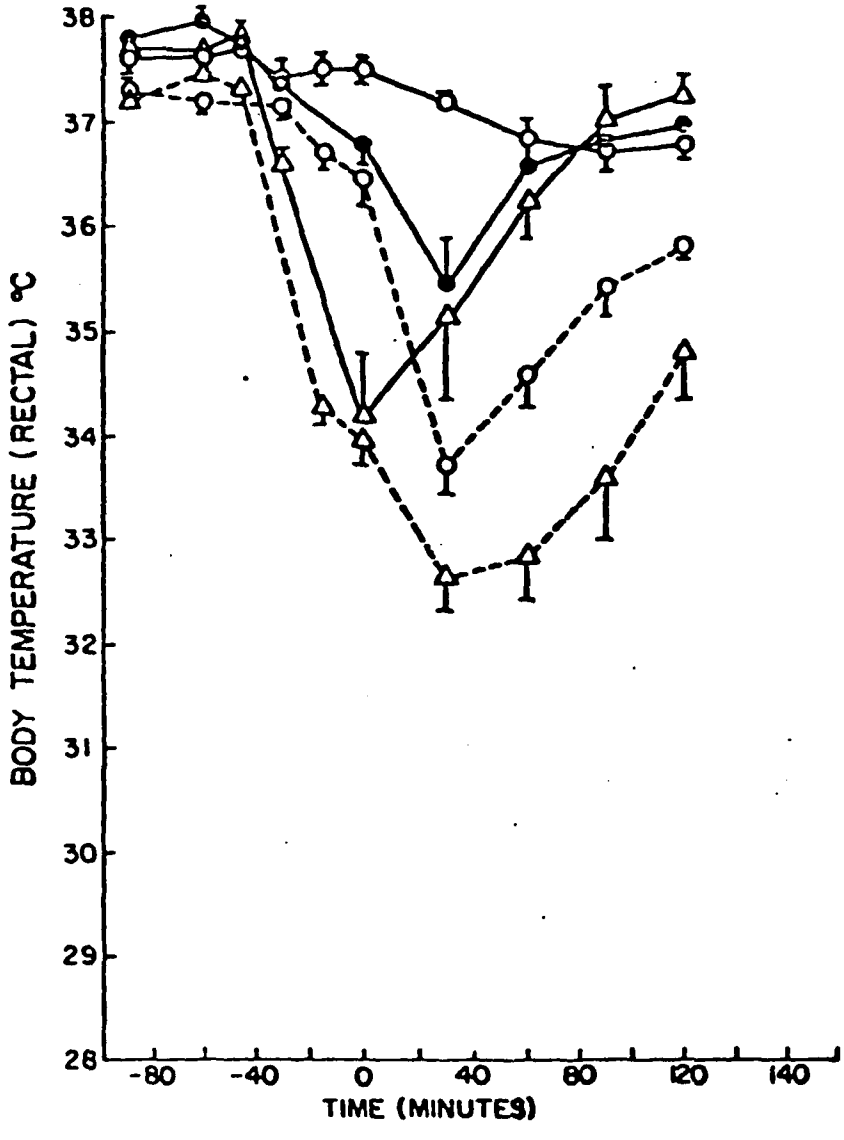

BODY TEMPERATURE (RECTAL) °C
38
37
36
35
34
33
32
31
30
29
28
-80
-40
0
40
80
120
140
TIME (MINUTES)

Fig 29

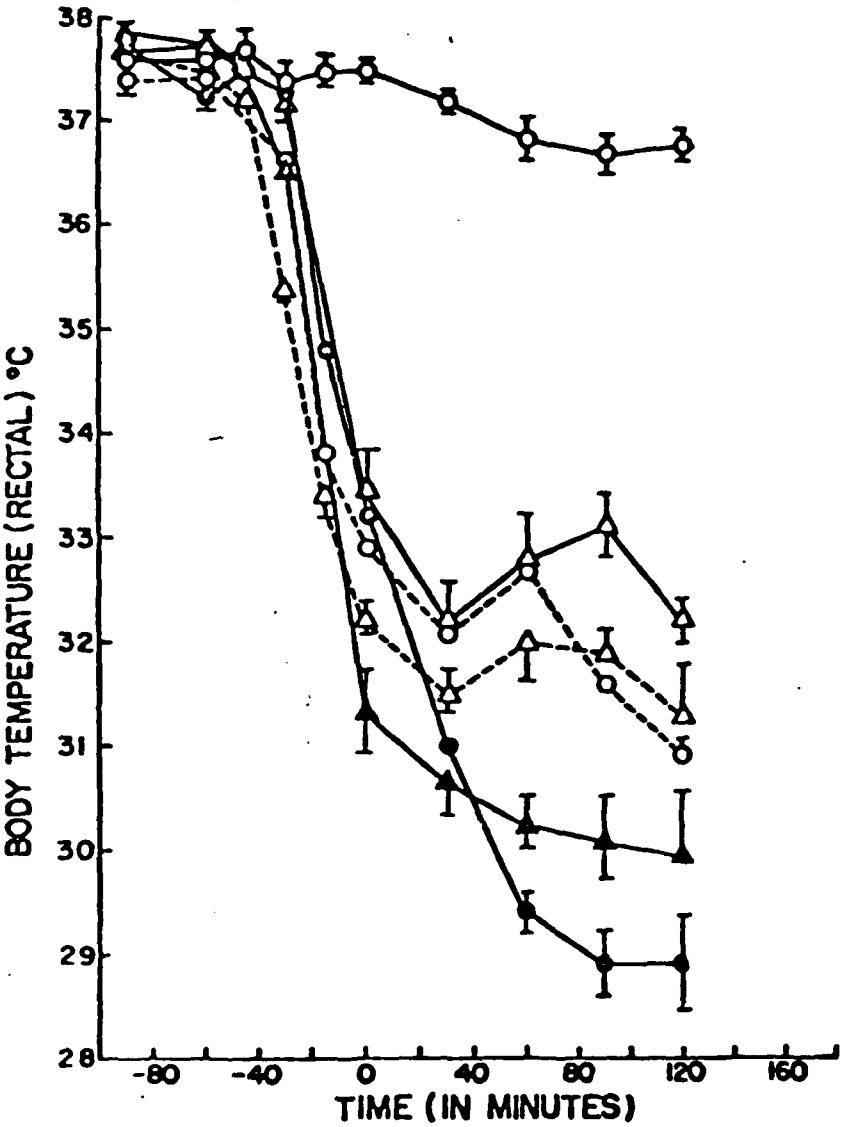

BODY TEMPERATURE (RECTAL) °C
38
37
36
35
34
33
32
31
30
29
28
-80
-40
0
40
80
120
160
TIME (IN MINUTES)

Fig 2b

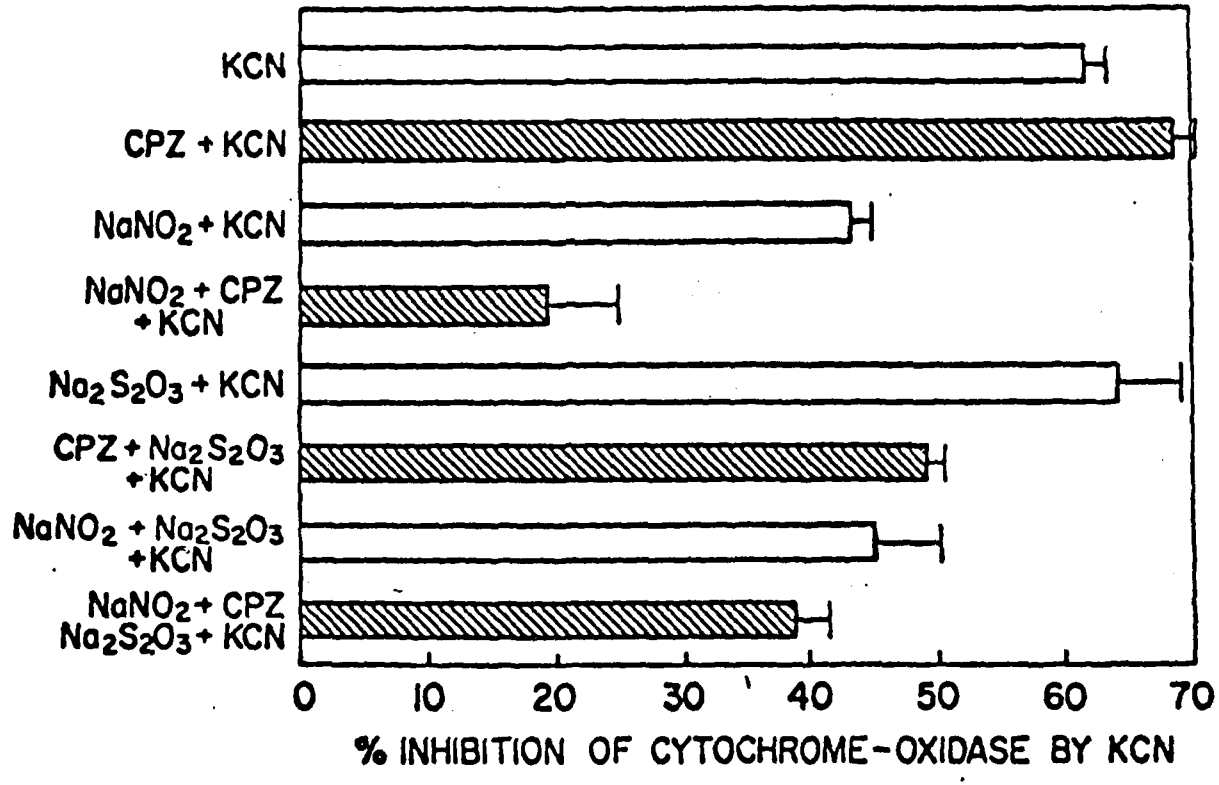

KCN
CPZ + KCN
NaNO2 + KCN
NaNO2 + CPZ + KCN
Na2S2O3 + KCN
CPZ + Na2S2O3 + KCN
NaNO2 + Na2S2O3 + KCN
NaNO2 + CPZ Na2S2O3 + KCN
0
10
20
30
40
50
60
70
% INHIBITION OF CYTOCHROME-OXIDASE BY KCN

Fig. 3

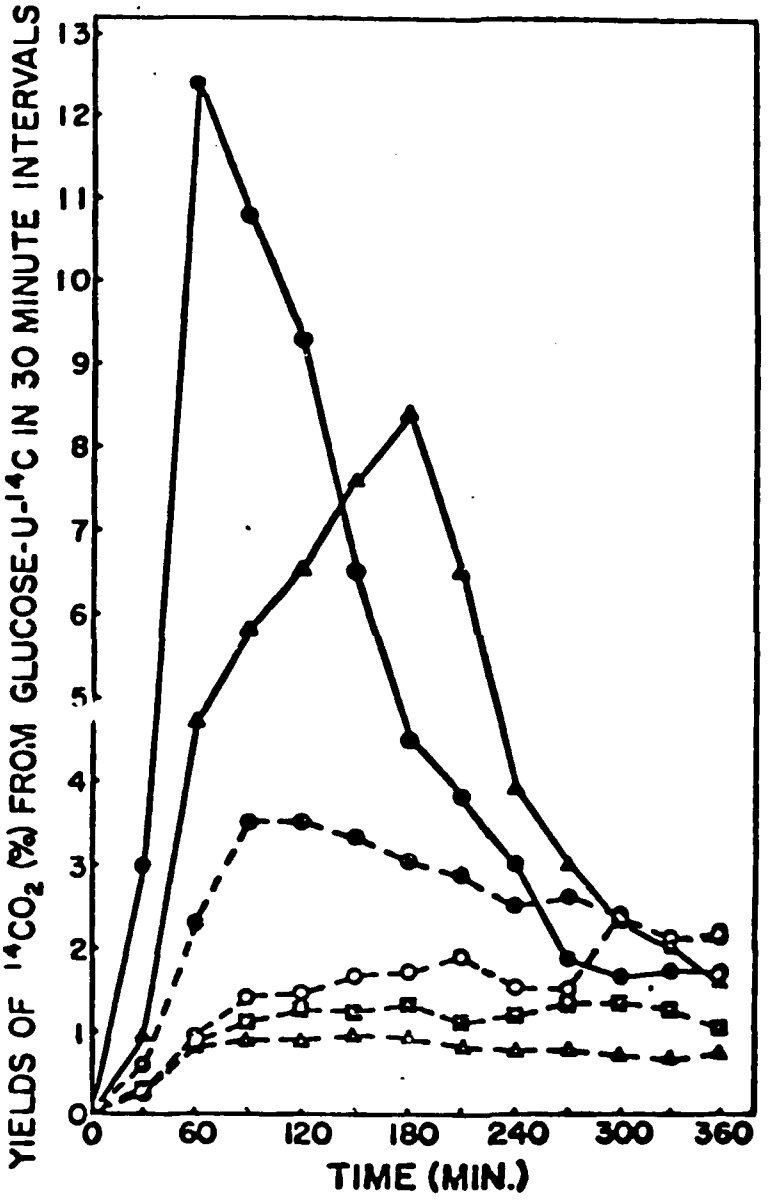

YIELDS OF $^{14}CO_2$ (%) FROM GLUCOSE-U-^{14}C IN 30 MINUTE INTERVALS
0
1
2
3
4
5
6
7
8
9
10
11
12
13
0
60
120
180
240
300
360
TIME (MIN.)

Fig 4

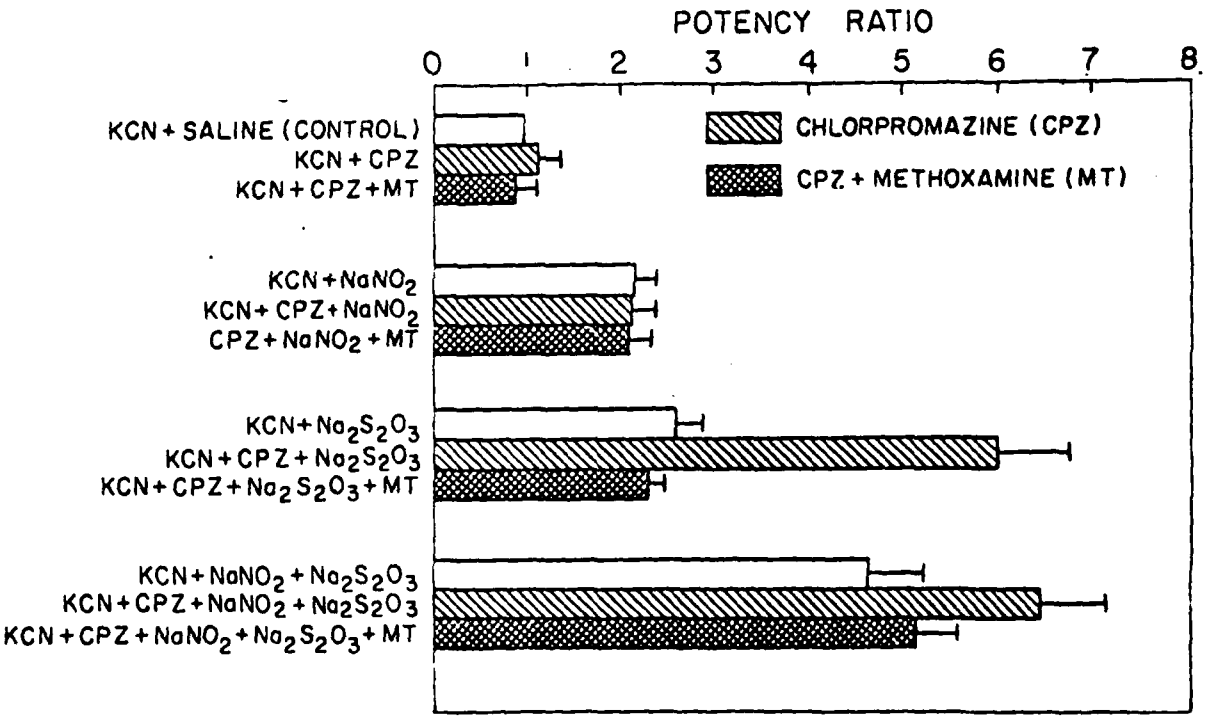

POTENCY RATIO
0
1
2
3
4
5
6
7
8
KCN + SALINE (CONTROL)
KCN + CPZ
KCN + CPZ + MT
KCN + NaNO2
KCN + CPZ + NaNO2
CPZ + NaNO2 + MT
KCN + Na2S2O3
KCN + CPZ + Na2S2O3
KCN + CPZ + Na2S2O3 + MT
KCN + NaNO2 + Na2S2O3
KCN + CPZ + NaNO2 + Na2S2O3
KCN + CPZ + NaNO2 + Na2S2O3 + MT
CHLORPROMAZINE (CPZ)
CPZ + METHOXAMINE (MT)

Fig 5

SECTION 2

Effect of Antagonists on the Physiological Disposition of Sodium Cyanide

There has been very little study on the effect of cyanide antagonists on the physiological disposition of cyanide. Investigation in this area may permit the gaining of greater insight into the selection of antidotal combinations. These studies have focused on the effects of oxygen and air either alone or in various combinations with sodium nitrite and/or sodium thiosulfate.

In cyanide intoxication the inhibition of cytochrome oxidase is believed to be the primary lethal effect (Keilin, 1930 Warburg, 1931). Since oxygen transport and tension are generally adequate, administration of oxygen should serve no useful purpose in antagonizing cyanide intoxication. However, oxygen has been shown to be of value in reversing some of the toxic effect produced by cyanide (Bernthal et al.,1928; Ivanov, 1959; Levine, 1959; Paulet, 1960: Cope, 1961; Skene et al., 1966) particularly if oxygen (Sheehy and Way, 1968) is administered in combination with sodium nitrite and sodium thiosulfate (Chen and Rose, 1952). Tolbert and Hughes (1959) indicated that approximately 10% of the radioactivity from tracer doses of $Na^{14}CN$ was recovered in the respired air and Gibson et al. (1969) reported that a high alveolar PO_2 would enhance the respiratory excretion of cyanide. These above observations suggest that oxygen may enhance the excretion of cyanide, especially when very high doses are employed.

The proposed study described herein was to evaluate the effects of O_2, $NaNO_2$, and $N_2S_2O_3$ either alone or in various combinations on the excretion of radioactive cyanide.

Materials

Compressed air and 100% oxygen were obtained from Industrial Air Products (Portland, OR). Carbon-14 labeled NaCN was purchased from the New England Nuclear Corporation (Boston, MA). This radioactive material was diluted with nonradioactive NaCN so that each animal received a tracer dose of approximately 5 μCi in a total dose of 5 mg/kg. Sodium nitrite and sodium thiosulfate were obtained from Fisher Scientific Company (Fairlawn, NJ). All chemicals employed in these studies were of the highest purity commercially available.

Methods

Male Swiss Webster mice weighing approximately 27 to 30 g were employed in these experiments. Radiorespirometry was conducted in a Roth Metabolism Chamber with minor modifications as described by Wang (1967). An organic trapping solution, ethanol-ethanolamine (2:1, v/v), was used to collect the radioactive compounds in the respiration gases. It should be pointed out that cyanide binds readily with most metals and therefore could not be determined accurately using a standard vibrating reed ionization chamber as has been employed by other investigators.

Pretreatment with air or O_2 and other antidotes was accomplished using a wooden container (24 x 12 x 12 inches) with a Plexiglass top and 2 rubber portholes to accommodate the operator's hands. Subsequent to the subcutaneous administration of $Na^{14}CN$, the mouse was placed in a glass respiration chamber. Respiratory gases produced were swept from the chamber through the trapping column which contained 20 ml of the trapping solution at a flow rate of 100 ml/min with air or 100% O_2. For the first 2 hours the trapping solution was collected at 10 minute intervals and for the final 1.5 hours it was collected

at 30 minute intervals. This trapping solution was withdrawn from the column by forcing it gently through a fritted glass disc into a 25 ml graduated cylinder. Absolute ethanol was added to make up for losses incurred through evaporation. A 5 ml aliquot of the trapping solution was added to 10 ml of scintillation fluid [4.0 g PPO, 0.3 g POPOP, and to toluene to make a volume of 1 litter (Wang, 1967)] and the radioactivity was determined in a Packard TriCarb Liquid Scintillation Counter (Model 3003). Aliquots of the trapping solution were acidified with N H_2 SO_4 and the gases were trapped in 0.5 N NaOH. The radioactive carbon dioxide was determined as $BaCO_3$ (Lindenbaum et al., 1948) and the remaining radioactivity in solution was determined by liquid scintillation. Less than 5% of the respiratory gas could be attributed to carbon dioxide.

Urine was collected under a dry ice ethanol mixture. The urine volumes were determined and 10 µl aliquots were added to 10 ml of Bray's Solution (Bray, 1960) and counted on the scintillation counter.

Immediately after completion of an experiment, the mouse was stunned and quickly frozen in an acetone-dry ice slurry, and the frozen carcass was stored at -190°C for later analysis. The frozen mice were homogenized for 5 minutes in 4 volumes of cold 0.2 M sodium carbonate buffer, pH 10. A 0.4 ml aliquot of this homogenate was added to 1.5 ml of tissue solubilizer (Protosol, New England Nuclear Corp.); incubated at 55°C for 60 minutes in a closed vial, and chilled. To this cold incubation mixture was then added 15 ml of scintillation fluid (4.0 gm PPO, 0.2 gm POPOP, 300 ml abs. ethanol, and toluene to make a volume of 1 liter) and the radioactivity was measured.

These experiments were carried out using 5 or more mice in each group. Air or O_2 was used either alone or in various combinations with $NaNO_2$ and/or $Na_2S_2O_3$. Oxygen (or air), $NaNO_2$ (100 mg/kg, i.p.), and $Na_2S_2O_3$ (1 g/kg, i.p.) were administered

60, 45, and 15 minutes respectively prior to the administration of NaCN (5 mg/kg, s.c.). The timing for the excretion of radioactivity began after cyanide administration. The area under the curve of the plot of the cumulative data was determined with a planometer.

The IBM 360 computer was programmed for the t test at a level of significance of 19/20 probability. This computer was interfaced with a Calcomp plotter accessory so that subsequent to the imput of the data, all construction drawings of the respiratory excretion curves were done solely by the Calcomp plotter. The level of significance between this mean of the different respiratory excretion curves, i.e., initial rate of excretion peak yield, area under the curve, cumulative yield, etc., were statistically analyzed by the computer. It should be pointed out that a different mouse is employed to obtain each respiratory excretion curve. Each curve presented graphically represents the mean of five or more individual curves. In other words, the data of each experiment were retrived from the memory core of the computer and the mean of five or more separate excretion curves was plotted by the computer interfaced with a Calcomp plotter. Almost simultaneously all the statistical parameters for the mean of each excretion curve were obtained and also cross comparison between all of the experimental groups were statistically analyzed by the computer.

RESULTS

All results are expressed as percent of total radioactivity recovered in order to compensate for small differences in the amount of radioactivity administered between the various experimental groups. Statistical comparisons of the mean radiorespirometric curves between O_2 and air with $NaNO_2$ and/or $Na_2S_2O_3$ in all possible experimental combinations were analyzed with an IBM 360 computer. However, in some instances, only a single illustration is presented as representative of all experimental groups.

The peak yield for the excretion of radioactivity in respired air after $Na^{14}CN$ administration varied between 40 and 80 minutes (Fig. 1). Oxygen significantly enhanced the respiratory excretion of radioactivity from $Na^{14}CN$ at the peak yield when compared with air either alone or in combination with $Na_2S_2O_3$. Furthermore, $Na_2S_2O_3$ accelerated the initial rate of excretion of radioactivity in the respired air in both air and oxygen treated animals, as the peak yield occurred at 40 minutes with $Na_2S_2O_3$ and at approximately 80 minutes without $Na_2S_2O_3$. It is important to note that during the ascending portion of the curve, administration of O_2 alone had very little influence on the initial rate of excretion of respiratory ^{14}C; however, a significant increase in the excretion continued after the peak yield in air was attained. The enhancement in the total excretion is best illustrated in Figure 2 by the cumulative recovery curves. It should be noted that $Na_2S_2O_3$ accelerated the initial rate, but not the total amount of radioactivity excreted, whereas O_2 increased the amount of radioactivity excreted, but did not affect the initial rate of excretion.

When $NaNO_2$ was employed (Fig. 3) either alone or with $Na_2S_2O_3$, the increase in rate and amount of excretion of radioactivity observed with $Na_2S_2O_3$ and O_2 alone respectively was not elicited, as no difference was noted between air and O_2 in the presence of $NaNO_2$.

Treatment with $NaNO_2$-$Na_2S_2O_3$ combination either with air or O_2 resulted in a marked decrease in the initial rate as well as the total amount of radioactivity excreted when compared with any other treatment group (Fig. 4). Only the data with oxygen are shown, but similar results were obtained with air in the four respective experimental groups. Although $Na_2S_2O_3$ accelerated the initial rate of excretion from $Na^{14}CN$ (Fig. 4.), the total amount of excretion was no greater than the group not receiving $Na_2S_2O_3$. The $NaNO_2$ may have caused a slight decrease in the amount of excretion; however, this decrease was not significant when compared with the O_2 alone or $Na_2S_2O_3$ groups. The total cumulative excretion of respiratory ^{14}C from $Na^{14}CN$ for all groups was approximately 4 to 5% of the administered dose and occurred predominantly during the first 120 minutes. The cumulative recovery of respiratory ^{14}C appears to be consistently greater in all groups given O_2 over the respective groups with air, but the difference was significant only with the group receiving either no pretreatment or with $Na_2S_2O_3$ alone.

Urinary excretion of radioactivity was greater than the respiratory excretion. Urine volumes were of nearly equal magnitude for all treatment groups for the 210 minute period. The total recovery of urinary radioactivity was approximately 7 to 9% of the dose administered and there were no differences noted between any of the experimental groups. With regard to the radioactivity retained in the body there also were no differences noted between experimental groups. The total recovery of the administered dose was approximately 92% to 103%.

DISCUSSION

The rate of respiratory excretion of cyanide is probably directly dependent upon the free cyanide level in biological fluids. At physiologic pH values, the cyanide would exist predominantly in the unionized form of HCN, because the pKa is 9.2 (Izatt et al., 1962); hence, it should be excreted relatively rapidly by the respiratory route. If the rate of respiratory excretion of radioactivity is dependent upon the tissue levels of free cyanide, then those treatment groups which decrease binding, for whatever reason, should have the most rapid rate of excretion of cyanide. It was somewhat surprising that those animals which received $Na_2S_2O_3$ alone should have a more rapid initial rate of respiratory excretion than those receiving only air or oxygen. This may be attributed to the fact that thiocyanate, the product of cyanide detoxication (Lang, 1932; Himwich and Saunders, 1948; Sorbo, 1953), is extensively bound with proteins (Pollay et al., 1966; Gibson et al., 1969; Pande and McMenamy, 1970). Sodium thiosulfate by virtue of enhanced thiocyanate production, may compete for cyanide binding sites, as both cyanide and thiocyanate are nucleophilic reagents, causing an increase in cyanide availability for respiratory excretion if it is displaced from these elec ron deficient sites. This proposal would be consistent with the work of Schubert and Brill (1968) which indicates the rapid action of $Na_2S_2O_3$ in the production of increase thiocyanate formation.

The respiratory elimination of cyanide has been examined by several investigators (Boxer and Rickards, 1952; Tolbert and Hughes, 1959; Friedberg and Schwarzkopf, 1969) and for cyanide alone our studies agree with most other studies. However, Tolbert and Hughes (1959) using rats administered $Na^{14}CN$ (1.7 mg/kg) recovered about 10% of the radioactivity in the respired

air and their results served as the basis for these studies. Unfortunately, it should be noted that in those studies, an ionization chamber was utilized to determine the radioactivity in the respired air. Our experience with such instrumentation indicates that cyanide binds avidly with the metal of the ion chamber thereby yielding erroneously high values.

The urinary and respiratory excretion of radioactive cyanide reflects approximately 13 percent of dose administered. Although this fraction may appear to be relatively small, it should be emphasized that the toxicity of cyanide probably resides in this labile or loosely bound cyanide pool rather than in the large inert bound cyanide or metabolite pool.

It should be pointed out that short term oxygen administration itself has very little effect on respiratory parameters (Watt, et al., 1943) and also appears to have minimal effects on the respiratory alterations produced by NaCN in dogs (Burrows et al., 1973). The increased respiratory excretion with oxygen alone may provide, in part, a basis for its enhanced antagonism of cyanide; however, other contributing mechanisms are essential to rationalize the more striking potentiation of oxygen with the sodium nitrite-sodium thiosulfate antidotal combination. It seems reasonable that the protective effect of oxygen probably reflects the algebraic sum of multiple protective mechanisms rather than a single one. Also these different mechanisms, although they may be minor by themselves, may potentiate each other such as in the interplay of oxygen, sodium nitrite and sodium thiosulfate. A question may be raised as to whether the enhanced respiratory excretion by oxygen, under certain conditions, contributes any role in antagonizing cyanide intoxication as the effects are not observed until forty to seventy minutes later and the lethal effects of cyanide can occur

sooner. In in vivo studies such as these, there is a time lag before the experimental effects become apparent. This would not be much different from the fact that there is a significant time lapse if one measures methemoglobin formation from sodium nitrite, and thiocyanate formation after sodium thiosulfate; yet, there has been little question raised as to these agents being effective cyanide antagonists, and that they exert their antidotal effect by binding and detoxifying cyanide.

In summary, the in vivo effect of various cyanide antagonists on the physiological disposition of cyanide has been presented, and the potential significance of these results in the antagonism of cyanide intoxication are discussed.

[1]Research was supported by Research Grants from the National Institutes of Health, U.S. Department of Health, Education and Welfare.

[2]Department of Physiological Sciences, Oklahoma State University, Stillwater, Oklahoma.

[3]Stanford Research Institute, Department of Fish Biology, Menlo Park, California.

REFERENCES

Bernthal, T. B., Bronk, D. W., Cordero, N., and Gesell, R. (1928). The regulation of respiration. XVIII. The effects of low and high alveolar oxygen pressure and of sodium cyanide on the carotid and femoral flow of blood as studied with the continuous electrometric method. Am. J. Physiol. 83:435-444.

Boxer, G. E. and Richards, J. C. (1952). Studies on the metabolism of the carbon of cyanide and thiocyanate. Arch. Biochem. 39:7-26, 1952.

Bray, G. A. (1960). A simple efficient liquid scintillator for counting aqueous solutions in a liquid scintillation counter. Analyt. Biochem. 1:279-285.

Burrows, E. G., Liu, D. H. W., and Way, J. L. (1973). Effect of oxygen on cyanide intoxication. V. Physiologic effects. J. Pharmacol. Exp. Ther. 184:739-748.

Chen, K. K. and Rose, C. L. (1952). Nitrite and thiosulfate in cyanide poisoning. J. Amer. Med. Assoc. 149:113-119.

Cope, C. (1961). The importance of oxygen in the treatment of cyanide poisoning. J. Am. Med. Assn. 175:1061-1064.

Friedberg, K. D. and Schwarzkopf, H. A. (1969). Blausaureexhalation bei der cyanidvergiftung, Arch. Toxicol. 24:235-248.

Gibson, Q. H., Parkhurst, L. J. and Geraci, G. (1969). The reaction of methemoglobin with some ligands. J. Biol. Chem. 244:4668-4676.

Himwich, W. A. and Saunders, J. P. (1948). Enzymatic conversion of cyanide to thiocyanate. Am. J. Physiol. 153:348-354.

Ivanov, K. P. (1959). The effect of elevated oxygen pressure on animals poisoned with potassium cyanide. Pharmacol. and Toxicol. (Russian, Translation, Consultants Bureau Inc.) 476-479.

Izatt, R., Christensen, J., Pack, R., and Bench, R. (1962). Thermodynamics of Metal-Cyanide Coordination. I pK, ΔH^0, and ΔS^0 Values as a Function of Temperature for Hydrocyanice Acid Dissociation in Aqueous Solution. Inorganic Chemistry 1:828-831.

Keilin, D. (1930). Cytochrome and intracellular oxidase. Proc. R. Soc. 106: 418-444.

Lang, K. (1932). Die rhodanbildung in tierkorper. Biochem. Z. 259:243-256.

Levine, S. (1959). Oxygen in the therapy of cyanide poisoning. J. Am. Med. Assn. 170:1585.

Lindenbaum, A., Schubert, J., and Armstrong, W. D. (1948). Rapid wet combustion method for Carbon determination (with particular reference to isotopic carbon). Anal. Chem. 20:1120.

Pande, C. S. and McMenamy, R. H. (1970). Thiocyanate binding with modified bovine plasma albumins. Arch. Biochem. Biophys. 13:260-267.

Paulet, G. (1960). L'intoxication cyanhydrique et son treatment, pp. 48-51. Masson and Co., Paris.

Pollay, M., Sevens, A., and Davis, C. (1966). Determination of plasma-thiocyanate binding and the Donnan ratio under simulated physiological conditions. Analyt. Biochem. 17:192-200.

Schubert, J., and Brill, W. A. (1968). Antagonism of experimental cyanide toxicity in relation to the in vivo activity of cytochrome oxidase. J. Pharmacol. Exp. Ther. 162:352-359.

Sheehy, M., and Way, J. L. (1968). Effect of oxygen on cyanide intoxication. III. Mithridate. J. Pharmacol. Exp. Ther. 161:163-168.

Skene, W. G., Norman, V. N., and Smith G. (1966). 3rd International Conference on Hyperbaric Medicine, ed. by I. W. Brown, Jr., and B. G. Cox, pp. 705-710. Nat. Acad. Sci. Washington D.C.

Sörbo, B. (1953). Crystalline rhodanese. I: Purification and physiochemical examination. Acta Chem. Scand. 7:1129-1136.

Tolbert, B. M. and Hughes, A. M. (1959). C^{14}-labeled cyanide: Radioactivity excretion in mice and estimation of radiation dose to humans. Metab. Clin. Exp. 8:73-78.

Wang, C. H. (1967). Radiorespirometry, Methods of Biochemical Analysis. Inter Science Publication. 15:311-368.

Warburg, O. (1931). Uber nicht-hemmung der zellatmung durch blaüsaure. Biochem. Z. 231:493-497.

Watt, J. G., Dunke, P. R., and Comroe, J. H. (1943). Effects of inhalation of 100% and 14% oxygen upon respiration of unanesthetized dogs before and after chemoreceptor denervation. Am. J. Physiol. 138:610-617.

LEGENDS

Figrue 1 Comparison of the respiratory excretion of radioactivity at 10 minute intervals between oxygen and air either alone or in combination with $Na_2S_2O_3$ (1 g/kg. i.p.) in mice receiving $Na^{14}CN$ (5 mg/kg. s.c.).

Figure 2 Cumulative respiratory excretion of radioactivity between air and oxygen alone or in combination with $Na_2S_2O_3$ (1 g/kg, i.p.) in mice receiving $Na^{14}CN$ (5 mg/kg, s.c.).

Figure 3 Comparison of the respiratory excretion of radioactivity in 10 minute intervals between oxygen or air with $NaNO_2$ (100 mg/kg. i.p.) either alone or in combination with $Na_2S_2O_3$ (1 g/kg, i.p.) in mice receiving $Na^{14}CN$ (5 mg/kg, s.c.).

Figure 4 Cumulative respiratory excretion of radioactivity between various combinations of O_2, $NaNO_2$ (100 mg/kg, i.p.) and $Na_2S_2O_3$ (1 g/kg, i.p.) in mice receiving $Na^{14}CN$ (5 mg/kg, s.c.).

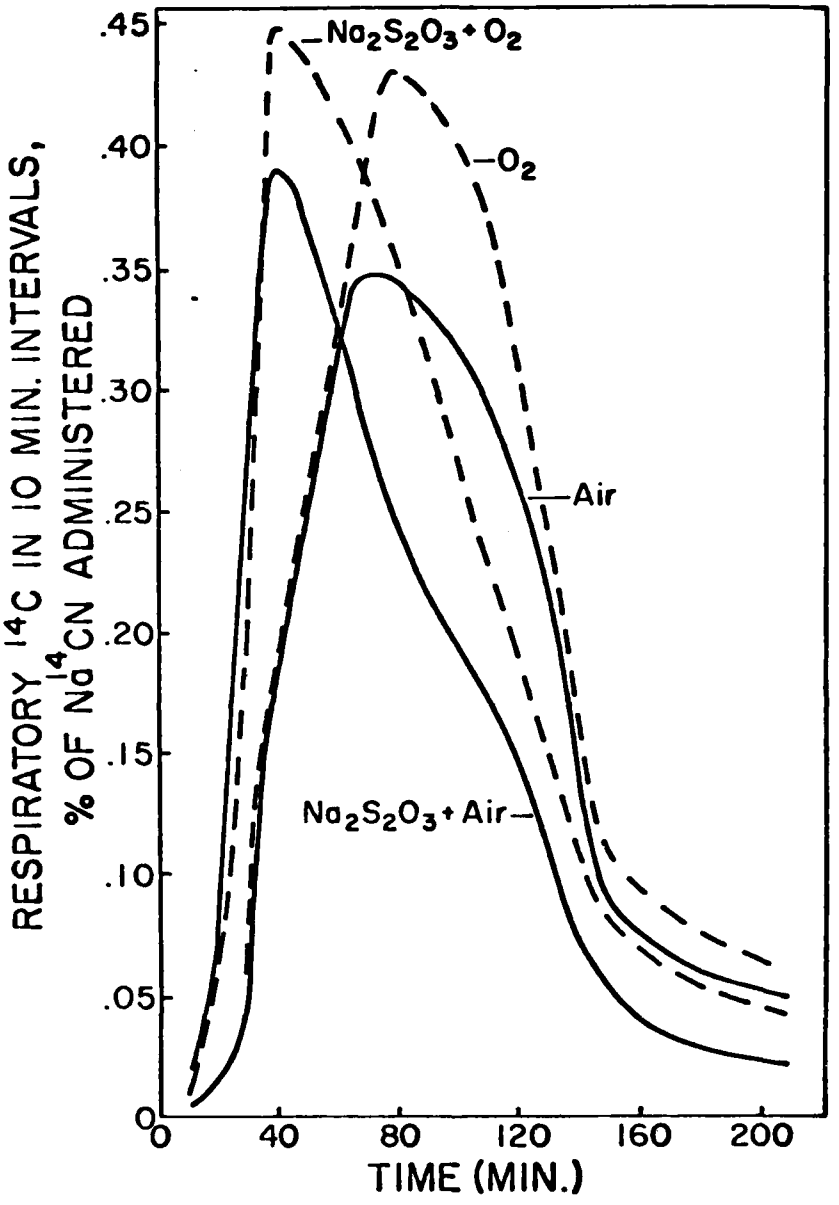

RESPIRATORY ^{14}C IN 10 MIN. INTERVALS,
% OF $Na^{14}CN$ ADMINISTERED
.45
.40
.35
.30
.25
.20
.15
.10
.05
0
0
40
80
120
160
200
TIME (MIN.)
$Na_2S_2O_3 + O_2$
O_2
Air
$Na_2S_2O_3$ + Air

Fig 1

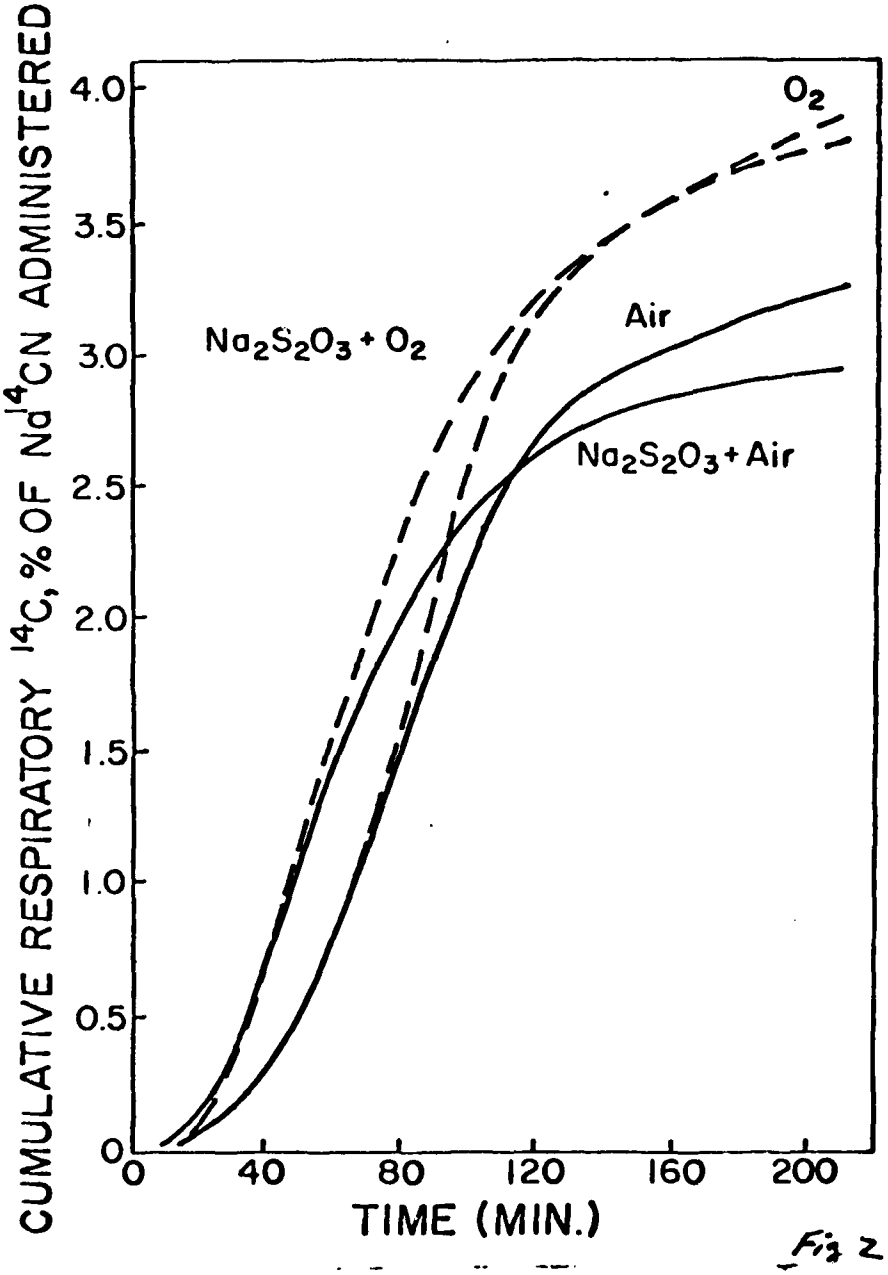

CUMULATIVE RESPIRATORY ^{14}C, % OF $Na^{14}CN$ ADMINISTERED
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0
0
40
80
120
160
200
TIME (MIN.)
O_2
$Na_2S_2O_3 + O_2$
Air
$Na_2S_2O_3$ + Air

Fig 2

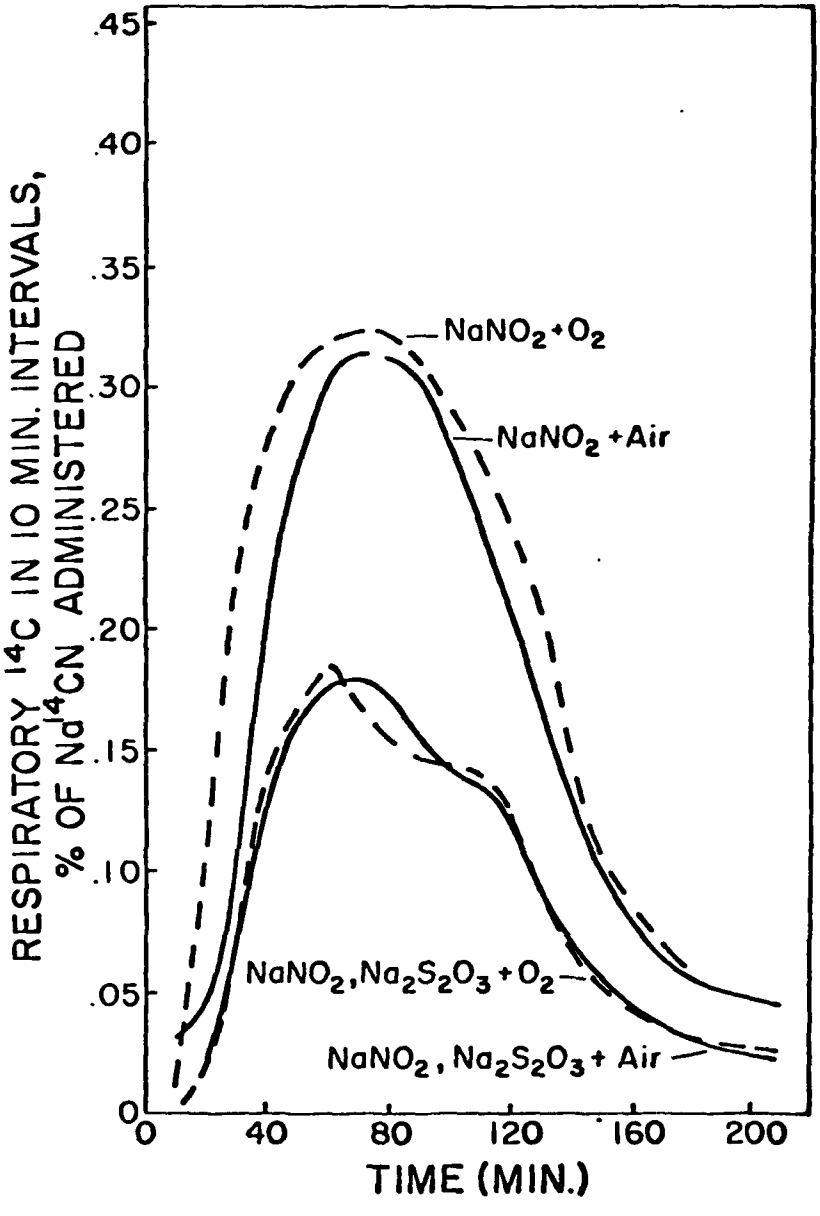

RESPIRATORY ^{14}C IN 10 MIN. INTERVALS, % OF $Na^{14}CN$ ADMINISTERED
.45
.40
.35
.30
.25
.20
.15
.10
.05
0
0
40
80
120
160
200
TIME (MIN.)
$NaNO_2 + O_2$
$NaNO_2$ + Air
$NaNO_2, Na_2S_2O_3 + O_2$
$NaNO_2, Na_2S_2O_3$ + Air

Fig 3

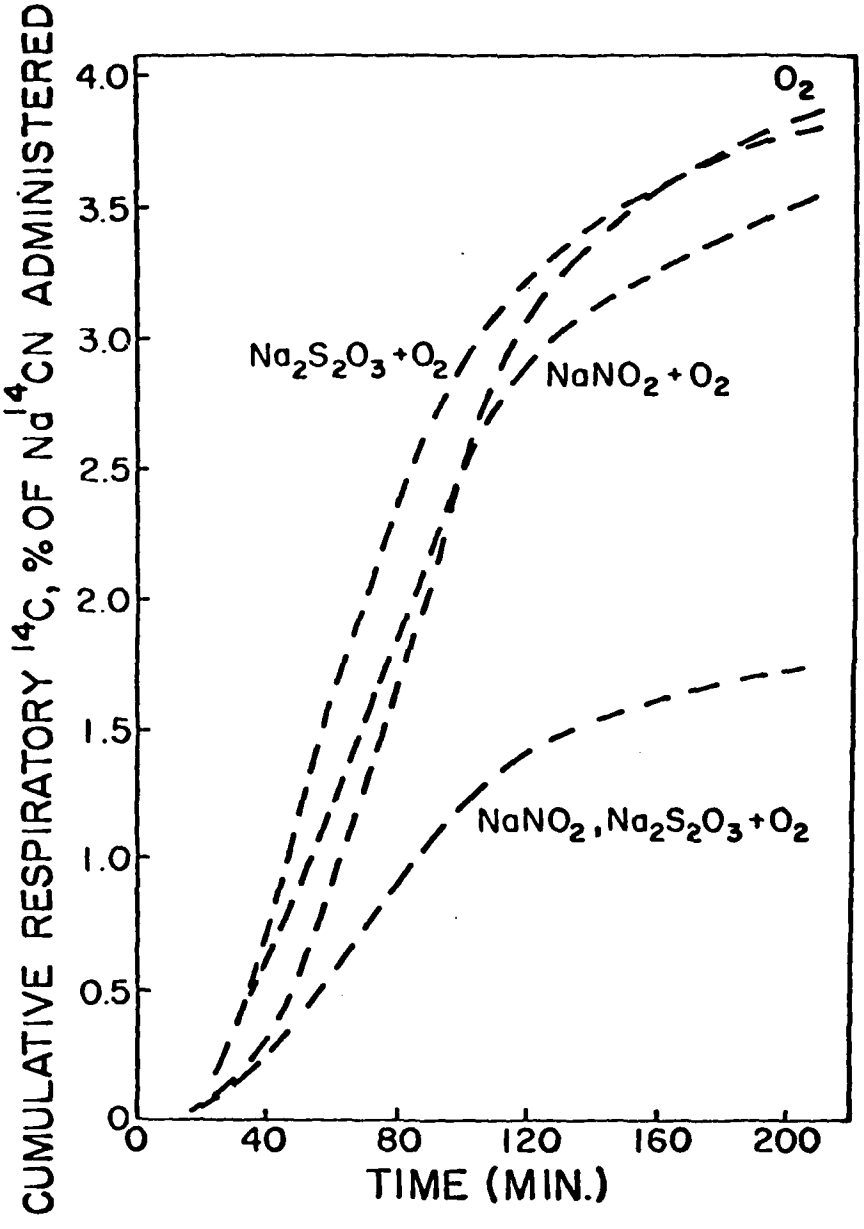

CUMULATIVE RESPIRATORY ^{14}C, % OF $Na^{14}CN$ ADMINISTERED
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0
0
40
80
120
160
200
TIME (MIN.)
O_2
$Na_2S_2O_3 + O_2$
$NaNO_2 + O_2$
$NaNO_2, Na_2S_2O_3 + O_2$

Fig 4

SECTION 3

Changes in Cyanide Response by Oxygen

In cyanide poisoning, oxygen utilization by the tissues is depressed due to inhibition of the respiratory enzyme, cytochrome oxidase (Keilin, 1930; Warburg, 1931); therefore, oxygen transport and oxygen tension are generally considered adequate, at least prior to the onset of the respiratory and cardiovascular collapse. Based on this reasoning, the administration of oxygen should serve no useful purpose in antagonizing cyanide intoxication. However, studies (Way et al., 1966 a, b; Sheehy and Way, 1968) have demonstrated that protection against cyanide intoxication in mice can be enhanced by the administration of oxygen, especially when oxygen is employed in combination with sodium nitrite and sodium thiosulfate.

Although there are numerous reports on the in vitro inhibition of cytochrome oxidase by cyanide, there have been very few studies conducted at the in vivo level. This is surprising, as the correlation of cyanide toxicity with its physiologic disposition and the rate of in vivo inhibition and reactivation of cytochrome oxidase are essential to evaluate the various antidotal combinations. Albaum et al (1946) have indicated that a lethal dose of cyanide will produce an in vivo inhibition of brain cytochrome oxidase. A more recent study has been reported (Schubert and Brill, 1968) which correlates the syndrome of cyanide poisoning and the effect of the classic cyanide antidotal combination of sodium nitrite and sodium thiosulfate with the rate of in vivo inhibition and reactivation of liver and spleen cytochrome oxidase.

The present study was initiated to investigate some of the biochemical changes involved in the protection afforded by oxygen in cyanide toxicity.

MATERIALS AND METHODS

Materials

The chemicals and their suppliers were as follows: potassium cyanide, sodium dithionate and potassium ferricyanide, Fisher Scientific Company (Fairlawn, NJ); cytochrome c (Type II, horse heart), Sigma Chemical Company (St. Louis, MO); Tris (grade A), Calbiochem (LaJolla, CA); reagent grade sodium-potassium tartrate, cupric sulfate, sodium nitrate, sodium thiosulfate, 38% formaldehyde solution, ferric nitrate, and potassium thiocyanate, J.T. Baker Company (Phillipsburg, NJ); practical grade p-benzoquinone , Eastman Kodak Company (Rochester, NY); dimethyl sulfoxide (DMSO), spectrophotometric grade, Aldrich Chemical Company (Milwaukee, WI); oxygen and nitrogen mixtures, Matheson company, Inc. (Rutherford, NJ); compressed air, Industrial Air Products Company (Portland, OR).

All solutions were prepared in deionized, double distilled water and were prepared immediately before use. The cyanide content of potassium cyanide was analyzed by the method of Liebig (Kolthoff and Sandell, 1943), and p-benzoquinone was purified by sublimation.

The chamber employed to administer oxygen and compressed air to the mice was constructed of 3/4-inch plywood (length, 24 inches; width, 12 inches; and height, 12 inches) with a 1/4-inch plexiglas top and two rubber portholes on the side which could be sealed. The chamber was relatively gas-tight and the rubber portholes were designed to fit snugly around the operator's arms. Oxygen concentration within the chamber was periodically monitored by means of a Mira Oxygen analyzer (Mira Corporation, Los Angeles, CA) and an oxygen concentration was maintained within 2% of the gas cylinder concentration in all parts of the chamber.

Cytochrome Oxidase Assay

Sixty minutes before administration of KCN, male Swiss-Webster mice (Horton Animal Laboratory, Oakland, CA) were placed in the treatment chamber which was equilibrated with the appropriate gas mixture. Sodium nitrite (100 mg/kg, subcutaneous) and sodium thiosulfate (1 g/kg, intraperitoneal) were administered 45 and 15 minutes, respectively, prior to KCN which was injected intraperitoneally in a volume corresponding to 1% of body weight. The animals were removed from the chamber at specific time intervals after KCN administration and sacrificed by decapitation. The liver was excised quickly, and excess blood was removed by blotting with filter paper. A 10% (w/v) crude liver homogenate was prepared in chilled distilled water (2°C) by use of a motor drive teflon tissue homogenizer and subsequently diluted to a 0.2% (w/v) liver homogenate with chilled (2°C) 0.03 M tris-HCL buffer, pH 7.4.

The incubation mixture contained one-half ml of cytochrome c solution (10^{-4} M, reduced with sodium dithionate) which was standardized by the method of Potter (1964), 2.4 ml of 0.03 M tris-HCL buffer, pH 7.4, and 0.1 ml of the 0.2% (w/v) liver homogenate. Analysis was conducted over a 5-minute period at 550 nm in a Beckman model DU spectrophotometer. Finally, 0.05 ml of saturated potassium ferricyanide solution was added to oxidize completely the cytochrome c and the optical density was redetermined. Cytochrome oxidase activity was expressed as the first order rate constant (Cooperstein and Lazarow, 1951; Smith, 1954). Protein concentration of the 0.2% (w/v) liver homogenate was determined by the method of Lowry (1951). The rate constant was expressed in terms of milligrams of protein per unit time (mg^{-1} sec^{-1}). The enzymatic activity of the liver homogenates were determined in duplicate and 4 or more animals were used per treatment group.

Brain cytochrome oxidase activity was determined with the following modifications. After removal of the brain, a 2% (w/v) crude homogenate was prepared in chilled distilled water (2°C) and diluted 10 fold with 0.02 M tris-HCL buffer, pH 7.4. Brain cytochrome oxidase activity then was determined as described for liver cytochrome oxidase.

Blood Cyanide Determination

Mice were pretreated in air or oxygen (95%) and administered sodium nitrite and sodium thiosulfate as described for the cytochrome oxidase determinations. Blood cyanide was determined by the method of Guilbault and Kramer (1965) with minor modifications (Isom and Way, 1975).

Plasma Thiocyanate Determination

Mice were treated and blood samples were collected as described for the blood cyanide determinations. A 0.3 ml plasma sample was transferred to a 15 ml corex centrifuge tube containing 1.0 ml of distilled water. Then 2.5 ml of ferric nitrate reagent [100 g $Fe(NO_3)_3$ $9H_2O$ and 200 ml HNO_3 (sp. g=1.10) diluted to one liter with distilled water] was added, and the mixture was centrifuged at 10,000 x g for 10 minutes in a Sorvall type RC-2 automatic refrigerated centrifuge (Ivan Sorvall, Inc., Norwalk, CT). Absorbance was recorded at 460 nm in a Beckman DU spectrophotometer (Beckman Instruments, Inc., Fullerton, CA) with a normal (untreated) plasma sample used as the reference. The determination of thiocyanate is based on the red color of the thiocyanate-ferric ion complex (Himwich and Saunders, 1948). Plasma thiocyanate concentration was then determined from a standard curve which was prepared daily.

Rhodanese Assay

The method of Sörbo (1955) was modified as follows: After the specific drug treatments, brain and liver tissues were removed from the animal as

rapidly as possible. A 10% (w/v) crude liver homogenate was prepared in 0.0125 M sodium thiosulfate by use of a motor driven teflon tissue homogenizer A 2% (w/v) homogenate was then prepared by transferring 5.0 ml of the 10% crude homogenate to a 25 ml volumetric flask and bringing the volume to 25 ml with ice cold 0.0125 M sodium thiosulfate. With respect to brain tissue, a 5% (w/v) homogenate was prepared with 0.0125 M sodium thiosulfate. Then 0.2 ml of the 2% (w/v) liver homogenate or 0.5 ml of the 5% (w/v) brain homogenate was added to an incubation mixture containing 1 ml $Na_2S_2O_3$ (0.125 M), 0.5 ml KH_2PO_4 (0.20 M) and 0.5 ml KCN (0.25 M) and shaken in a water bath for 5 minutes at 37.5°C in stoppered 25 ml erlenmeyer flasks. The reaction was stopped with 0.5 ml of 38% formaldehyde solution. Then 2.5 ml of ferric nitrate reagent was added and subsequently diluted with 20 milliliters of distilled water and centrifuged at 6,000 x g for 10 minutes. The absorbance was recorded at 460 nm with water in the reference cuvette, and the enzymatic activity was expressed as mg of cyanide converted to thiocyanate per gram of tissue. Standard curves for brain and liver were determined by adding known amounts of potassium thiocyanate to the incubation mixture containing formaldehyde.

Statistical Analyses

Standard errors and unpaired Student's t test were computed on a Wang 600 programmable calculator (Wang Laboratories, Tewksburg, MA). A probability level of 0.05 was used in testing for significant differences between experimental groups.

RESULTS

Liver cytochrome oxidase activity was measured in mice pretreated in air or oxygen (95%) at various time intervals after the intraperitoneal administration of KCN, 5 mg/kg (Figure 1). Inhibition of liver cytochrome oxidase occurred very rapidly and within 2 minutes enzymatic activity was inhibited approximately 75%. Maximum inhibition occurred at 5 minutes after cyanide administration in both the air and oxygen treatment groups. The differences observed between air and oxygen pretreatment became quite noticeable at 15 minutes after cyanide administration, and the animals maintained in oxygen returned to control values approximately 10 minutes prior to mice maintained in air.

Mice treated in air and administered a sublethal dose of KCN, 5 mg/kg, exhibited a rapid respiratory stimulation followed by agitation, lack of coordination, and convulsions. The severity of these symptoms seemed to parallel the inhibition of cytochrome oxidase. On the other hand, these behavioral patterns were not evident in animals administered KCN, 5 mg/kg, and maintained in oxygen (95%).

The symptoms exhibited by the air treated animals were dose-dependent and were barely detectable at one and 2 mg/kg KCN. However, animals administered 3, 4, and 5 mg/kg KCN displayed gasping, irregular breathing, and convulsions with an onset of about 2 to 3 minutes post cyanide administration. Mice receiving 6 mg/kg KCN exhibited severe convulsions with cessation of respiration and death occurring in 20% of the animals. Doses

higher than 6 mg/kg KCN resulted in death to all animals within 4 minutes after administration of cyanide. The oxygen-treated mice displayed these behavioral patterns only at the 6 mg/kg dose and the symptoms were not as severe and were of a shorter duration than the air-treated experimental group.

The effect of varying the treatment concentration of oxygen on liver cytochrome oxidase activity after administration of nonlethal doses of cyanide was determined as shown in Table 1. The rate constant, k, determined in animals treated with KCN (2mg/kg) showed little difference from that of the control. However, the enzymatic activities of all treatment groups at 3 and 4 mg/kg were significantly different from controls. The rate constants determined at 11% oxygen for the 3 and 4 mg/kg doses were considerably lower than those of higher concentrations of oxygen. The effect of oxygen treatment was most noticeable at KCN dose of 4 mg/kg at which the enzymatic activity increased from 22% to 66% of that of the controls as the oxygen concentration was increased from 11% to 95%.

In order to assess the ability of sodium nitrite and sodium thiosulfate to alter the symptoms of cyanide poisoning, these antidotes were administered 45 and 15 minutes, respectively, before KCN (Figure 2). It was quite surprising to find that in animals pretreated with sodium nitrite and sodium thiosulfate, liver cytochrome oxidase activity at 3 minutes post cyanide administration was not significantly different from the control in spite of the fact that the animals were dead. In contrast, brain cytochrome oxidase activity was inhibited in animals treated with the antidotes. In mice receiving a lethal dose of cyanide (10 mg/kg), but no antidote, both brain and liver enzymatic activities were significantly less than those of the controls.

Since the liver contains a high concentration of rhodanese, the possibility exists that this enzyme metabolizes cyanide in the tissue homogenates in the presence of its substrate, sodium thiosulfate, thereby resulting in cytochrome oxidase reactivation. Therefore, 10 mM of sodium sulfite, a rhodanese inhibitor (16) was added to the homogenate immediately after preparation. The results obtained under these conditions were essentially similar to those obtained in Figure 2.

Brain cytochrome oxidase activity was studied in animals pretreated with air or oxygen (95%), sodium nitrite and sodium thiosulfate (Figure 3). Pretreatment time for air and oxygen was 60 minutes and KCN was administered at a dose of 45 mg/kg. Enzymatic activity was rapidly inhibited and maximum inhibition of both the air and oxygen groups was observed at 2 minutes after cyanide administration. Oxygen treatment did not appear to reactivate cytochrome oxidase in the brain more rapidly that did air. Significant differences between the two treatment groups were detected only at 20 and 60 minutes post cyanide administration. It is of interest to note that reactivation of cytochrome oxidase in the brain (Figure 3) occurred at a much slower rate than in the liver (Figure 1). The symptoms of cyanide poisoning were detectable only in the air treated animals and were the same as described previously but of shorter duration.

Brain cytochrome oxidase activity in mice which received a gradient dose of KCN ranging from 5 to 85 mg/kg is shown in Figure 4. These animals were pretreated with sodium nitrite and sodium thiosulfate and enzymatic activity was determined 10 minutes after KCN. Treatment with oxygen resulted in a marked shift to the right in the curves representing the lines of best fit as determined by log-probit analysis (Figure 4). All points on the oxygen curve were significantly different from the air treatment curve. The dose of KCN required to produce 50% inhibition of brain

cytochrome oxidase was increased from 24 (16.6-34.8) mg/kg in the air treatment group to 55 (39.3-77.0) mg/kg in the animals placed in an oxygen atmosphere. The slopes of the two curves were not significantly different as determined by log-probit analysis. Mice treated with oxygen displayed the cyanide intoxication syndrome only at 75 and 85 mg/kg and death occurred at 90 mg/kg KCN. The air treated animals exhibited agitation, lack of coordination and depressed respiration at 25 and 25 mg/kg KCN. Convulsions were observed at 45 mg/kg in the air treatment group, and death occurred at 55 mg/kg KCN.

Blood cyanide levels were determined in animals receiving KCN, 45 mg/kg after pretreatment with air or oxygen (95%) and sodium nitrite-sodium thiosulfate (Figure 5). Cyanide blood levels increased rapidly and maximum levels were observed at 5 minutes after KCN injection. Blood cyanide decreased slowly both in the air and oxygen treatment groups. However, no significant differences were noted except at the 15 and 60 minute intervals. It is of interest to note that the decline of cyanide blood levels of air and oxygen groups parallels the inhibition of brain cytochrome oxidase (Figure 4).

Rhodanese activity of brain and liver was then compared on a time basis in mice pretreated with the antidotes and receiving 45 mg/kg KCN. Control rhodanese activity of the liver was approximately 15 times greater than brain in both air and oxygen, and no significant difference in rhodanese activity could be detected between air and oxygen treatment at the various time periods after KCN injection. When rhodanese activity was determined 10 minutes after administering gradient doses (5 to 45 mg/kg) of potassium cyanide, no significant differences could be detected between air and oxygen treatment in brain and liver rhodanese activity.

DISCUSSION

It should be pointed out that these studies have been conducted with fairly high doses of cyanide, as this approach has been successful in eliciting some biochemical and physiological differences between oxygen and air (Burrows et al., 1973; Isom and Way, 1974 and 1976).

It has been demonstrated that oxygen and air treated mice displayed the same degree of cytochrome oxidase inhibition during the first 5 minutes after administration of sublethal doses of cyanide, yet only the air group exhibited the symptoms of cyanide intoxication. These results were unexpected since Schubert and Brill (1968) indicated that the symptoms of cyanide intoxication paralleled cytochrome oxidase inhibition. The appearance and disappearance of these effects are reported to be dose dependent (Schubert and Brill, 1968). These possible differences between oxygen and air may be dose related since numerous investigators have shown that certain physiological changes induced by low cyanide concentrations are sensitive to oxygen (Brodie, 1959; Cope, 1961; Fernandez et al., 1963; Skene et al., 1966), whereas it does not alter the physiological changes induced by higher cyanide doses (Burrows et al., 1973). Also, the difference observed between air and oxygen during the first 5 minutes after cyanide administration may be explained by oxygen activation of cyanide-resistant respiratory pathways, thereby bypassing the cyanide blockade of cytochrome oxidase. Studies have shown that exposure to sublethal doses of cyanide will divert changes in carbohydrate intermediary metabolism to pathways which are less sensitive to cyanide (Isom and Way, 1974).

The concentration of oxygen employed in the treatment of cyanide intoxication appears to be important in the reactivation of cytochrome oxidase. As the concentration of oxygen is increased from 11 to 100% at 4 mg/kg KCN, an increase in cytochrome oxidase activity was noted which is in agreement with

lethality studies (Sheehy and Way, 1968).

It is interesting to note that the liver cytochrome oxidase activity from mice pretreated with the sodium nitrite-sodium thiosulfate combination and then receiving a lethal dose of cyanide (70 mg/kg) was the same as those animals which received the antidotal combination, but did not receive cyanide. This is in contrast to brain tissue as cyanide did inhibit cytochrome oxidase under these conditions. This dissociation between the results of liver and brain cytochrome oxidase enzymatic activity may be attributed to various factors: Firstly, the disposition of cyanide to liver cytochrome oxidase may be minimal due to high content and turnover number of rhodanese in the liver (Sörbo, 1951). Secondly, when cyanide has inhibited liver cytochrome oxidase, the complex formed is readily dissociable. Therefore, a coupled enzymatic reaction between cytochrome oxidase and rhodanese can lead to a rapid reactivation of cytochrome oxidase due to cyanide biotransformation to thiocyanate. Thirdly, the distribution of the active form of the cyanide antidotal combination, sodium thiosulfate, and nitrite generated methemoglobin, is very limited in brain tissues, thereby resulting in the brain's higher sensitivity to cyanide. A preliminary communication of these results was reported earlier (Isom and Way, 1976). In view of these findings, determination of brain rather than liver cytochrome oxidase would serve as a more appropriate model in evaluation of antidotal therapy against cyanide intoxication.

It is of interest to note that the reactivation of brain cytochrome oxidase in the presence of sodium nitrite and sodium thiosulfate corresponds with the decline of cyanide blood levels but not much difference was noted between oxygen and air.

REFERENCES

Albaum, H. G., Tepperman, J. and Bodansky, O.: The *in vivo* inactivation by cyanide of brain cytochrome oxidase and its effect on glycolysis on the high energy phosphorous compounds in brain. J. Biol. Chem. 164:45-51, 1946.

Brodie, D. A.: The effect of thiopental and cyanide on the activity of inspiratory neurons. J. Pharmacol. Exp. Ther. 126:264-269, 1959.

Burrows, G. E., Liu, C. H. W. and Way, J. L.: Effect of oxygen on cyanide intoxication. V. Physiological effects. J. Pharmacol. Exp. Therap. 184:739-748, 1973.

Cooperstein, S. J. and Lazarow, A.: A microspectrophotometric method for the determination of cytochrome oxidase. J. Biol. Chem. 189:665-670, 1951.

Cope, C.: The importance of oxygen in the treatment of cyanide poisoning. J. Am. Med. Ass. 175:1061-1064, 1961.

Fernandez, A. F., Gonzalez-Quintata, J. and Russek, M.: Effect of low concentrations of cyanide on Q_{O_2} of tissue slices. Am. J. Physiol. 204:314-316, 1963.

Guilbault, G. G. and Kramer, D. N.: Specific detection of cyanide using various quinone derivatives. Anal. Chem. 37:1395-1399, 1965.

Himwich, W. A. and Saunders, J. P.: Enzymatic conversion of cyanide to thiocyanate. Am. J. Physiol. 153:348-354, 1948.

Isom, G. E. and Way, J. L.: Effect of oxygen on cyanide intoxication. VI. Reactivation of cyanide-inhibited glucose metabolism. J. Pharmacol. Exp. Therap. 189:235, 1974.

Isom, G. E. and Way, J. L.: Effect of sublethal doses of cyanide on glucose catabolism. Biochem. Pharmacol. 24:871, 1975.

Isom, G. E. and Way, J. L.: Lethality of cyanide in the absence of inhibition of liver cytochrome oxidase. Biochem. Pharmacol. 25:605-608, 1976.

Keilin, D.: Cytochrome and intracellular oxidase. Proc. R. Soc. 106:418-444, 1930.

Kolthoff, I. M. and Sandell, E. B.: Textbook of Quantitative Inorganic Analysis, revised ed., The MacMillan Company, N. Y., 1943.

Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J.: Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193:265-275, 1941.

Potter, V. R.: Preparation and Standardization of cytochrome c. In Manometric Techniques, ed. by Umbreit, W. W., Burris, R. H. and Stouffer, J. F., 270-273, Burgess Publishing Company, Minneapolis, 1964.

Schubert, J. and Brill, W. A.: Antagonism of experimental cyanide toxicity in relation to the *in vivo* activity of cytochrome oxidase. J. Pharmacol. Exp. Ther. 162:352-359, 1968.

Sheehy, M. and Way, J. L.: Effect of oxygen on cyanide intoxication. III. Mithridate. J. Pharmacol. Exp. 161:163-168, 1968.

Skene, W. G., Norman, J. N. and Smith, G.: Effect of Hyperbaric oxygen in cyanide poisoning. In Hyperbaric Medicine, ed. by Brown, I. W. and Cox, B. G., 705-710, publication 1404, National Academy of Sciences, 1966.

Smith, L.: Spectrophotometric assay of cytochrome c oxidase. In Methods of Biochemical Analysis, ed. by Glick, D., 427-434, Academic Press, N. Y., 1954.

Sörbo, B. O.: On the properties of rhodanese. Partial purification, inhibitors and intracellular distribution. Acta Chem. Scand. 5:724-734, 1951.

Sörbo, B. O.: Rhodanese. In Methods in Enzymology, ed, by Colowick, S. P. and Kaplan, N. O. 2:334-337, Academic Press Inc., New York, 1955.

Warburg, O.: Uber Nicht-Hemmung der Zellatmung durch Blausaure. Biochen. Z. 231:493-497, 1931.

Way, J. L., Gibbon, S. L. and Sheehy, M.: Cyanide intoxication: Protection with oxygen. Science (Washington) 152:210-211, 1966a.

Way, J. L., Gibbon, S. L. and Sheehy, M.: Effect of oxygen on cyanide intoxication. I. Prophylactic protection. J. Pharmacol. Exp. Ther. 153:381-385, 1966b.

Legends for Figures

Figure 1. Liver cytochrome oxidase activity determined after the intraperitoneal administration of potassium cyanide (5 mg/kg). ● - ●, mice treated in air; O - O, mice treated in oxygen. Each point represents the average value ± S.E.M. from four or more animals

Figure 2. Liver and brain cytochrome oxidase activity measured 3 minutes after the administration of lethal doses of cyanide. A- control (saline); B- KCN (10 mg/kg, i.p.); C- KCN (70 mg/kg, i.p.) administered to mice pretreated with sodium nitrite (100 mg/kg, s.c.) and sodium thiosulfate (1 g/kg, i.p.). Each value represents the average ± S.E.M. from four or more animals. *Significantly different from control at 0.05 probability level.

Figure 3. Brain cytochrome oxidase activity after the administration in air of potassium cyanide (45 mg/kg, i.p.) to mice pretreated with sodium nitrite (100 mg/kg, s.c.) and sodium thiosulfate (1 g/kg, i.p.). ● - ●, mice treated in air; O - O mice treated in oxygen. Each point represents the average ± S.E.M. from four or more animals.

Figure 4. Brain cytochrome oxidase activity measured 10 minutes after the administration of gradient doses of potassium cyanide. ● - ●, mice treated in air; O - O, mice treated in oxygen. Each point represents the average value from four or more animals.

Figure 5. Blood levels of cyanide after the intraperitoneal administration of potassium cyanide (45 mg/kg, i.p.) to mice pretreated with sodium nitrite (100 mg/kg, s.c.) and sodium thiosulfate (1 g/kg). ● - ●, mice treated in air O - O, mice treated in oxygen. Each point represents the average value ± S.E.M. from four or more animals.

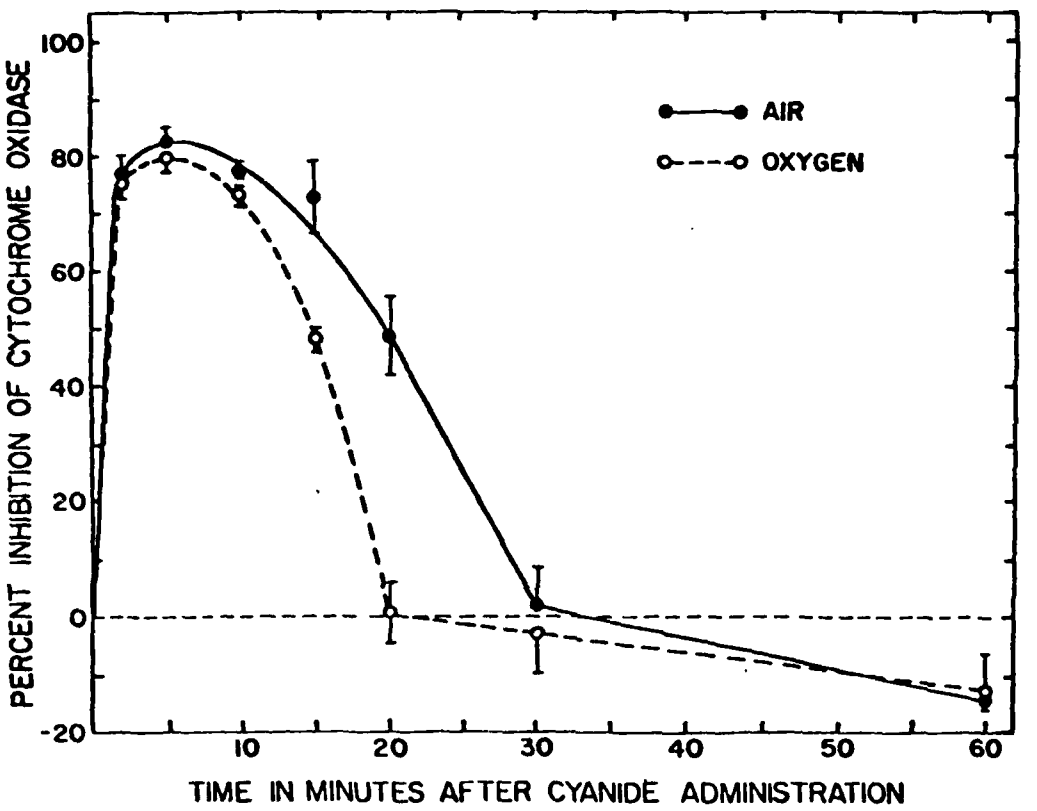

PERCENT INHIBITION OF CYTOCHROME OXIDASE
100
80
60
40
20
0
-20
10
20
30
40
50
60
TIME IN MINUTES AFTER CYANIDE ADMINISTRATION
AIR
OXYGEN

Fig. 1

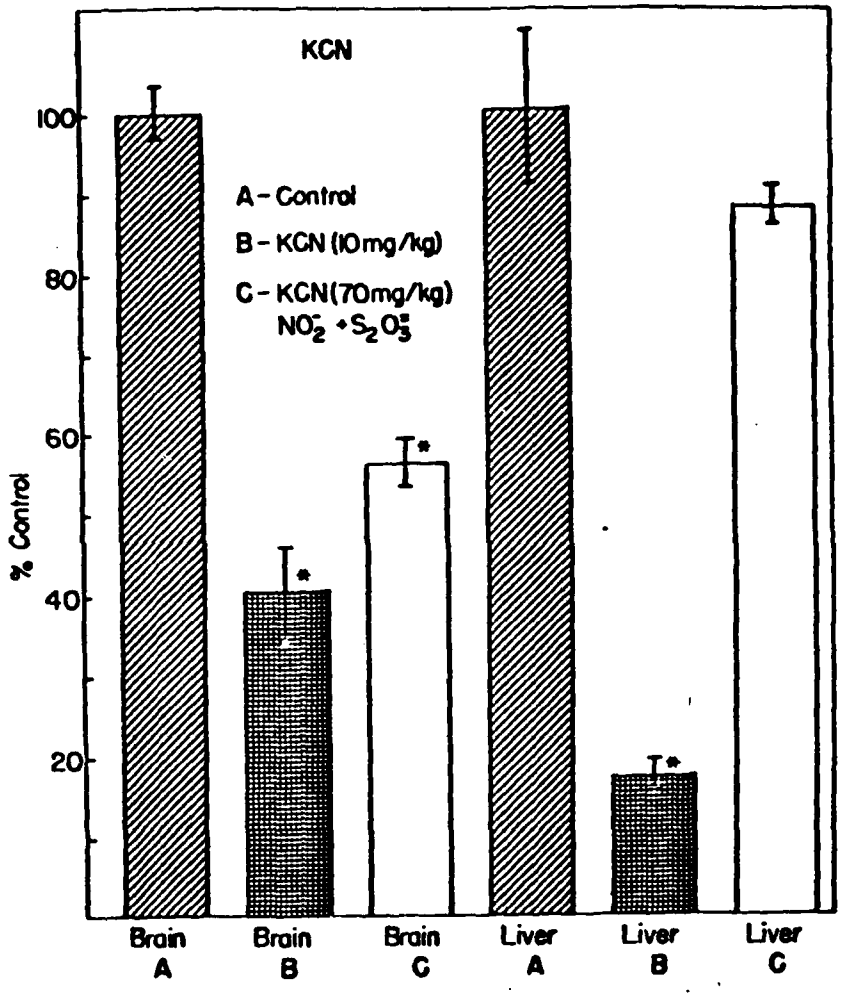

KCN
100
80
60
40
20
% Control
A - Control
B - KCN (10mg/kg)
C - KCN(70mg/kg)
$NO_2^- + S_2O_3^=$
*
*
*
Brain A
Brain B
Brain C
Liver A
Liver B
Liver C

Fig. 2

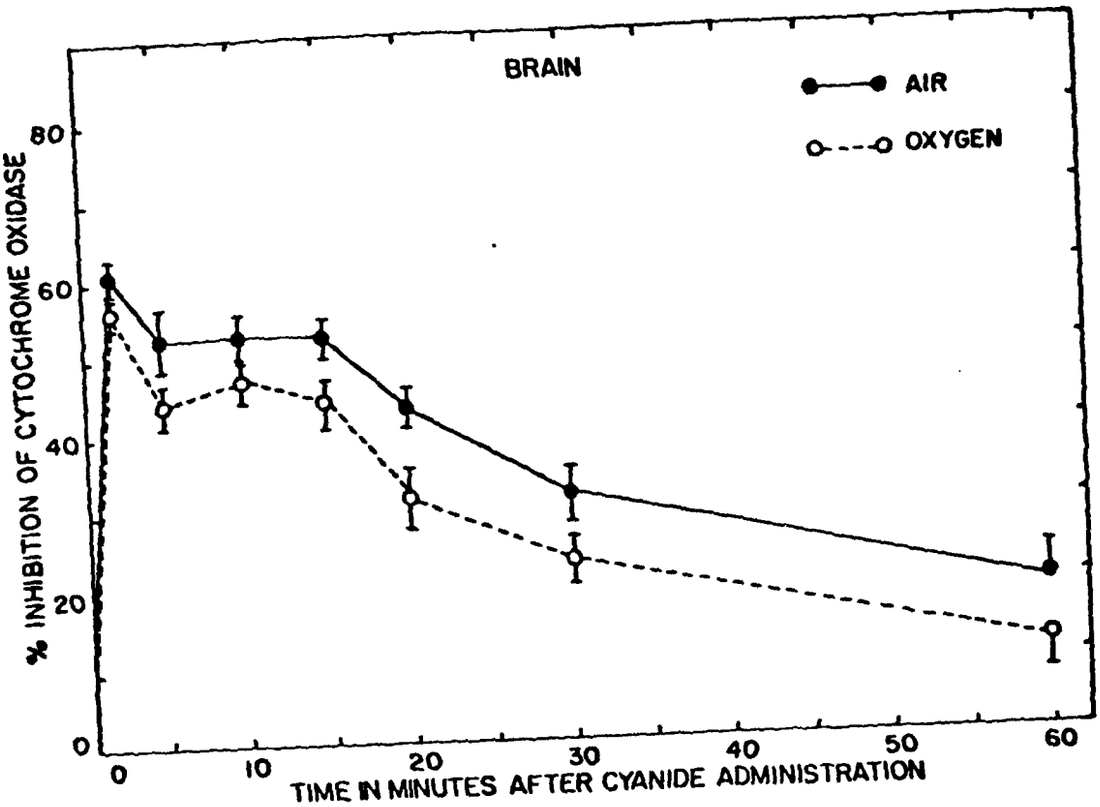

BRAIN
AIR
OXYGEN
% INHIBITION OF CYTOCHROME OXIDASE
0
20
40
60
80
0
10
20
30
40
50
60
TIME IN MINUTES AFTER CYANIDE ADMINISTRATION

Fig. 3

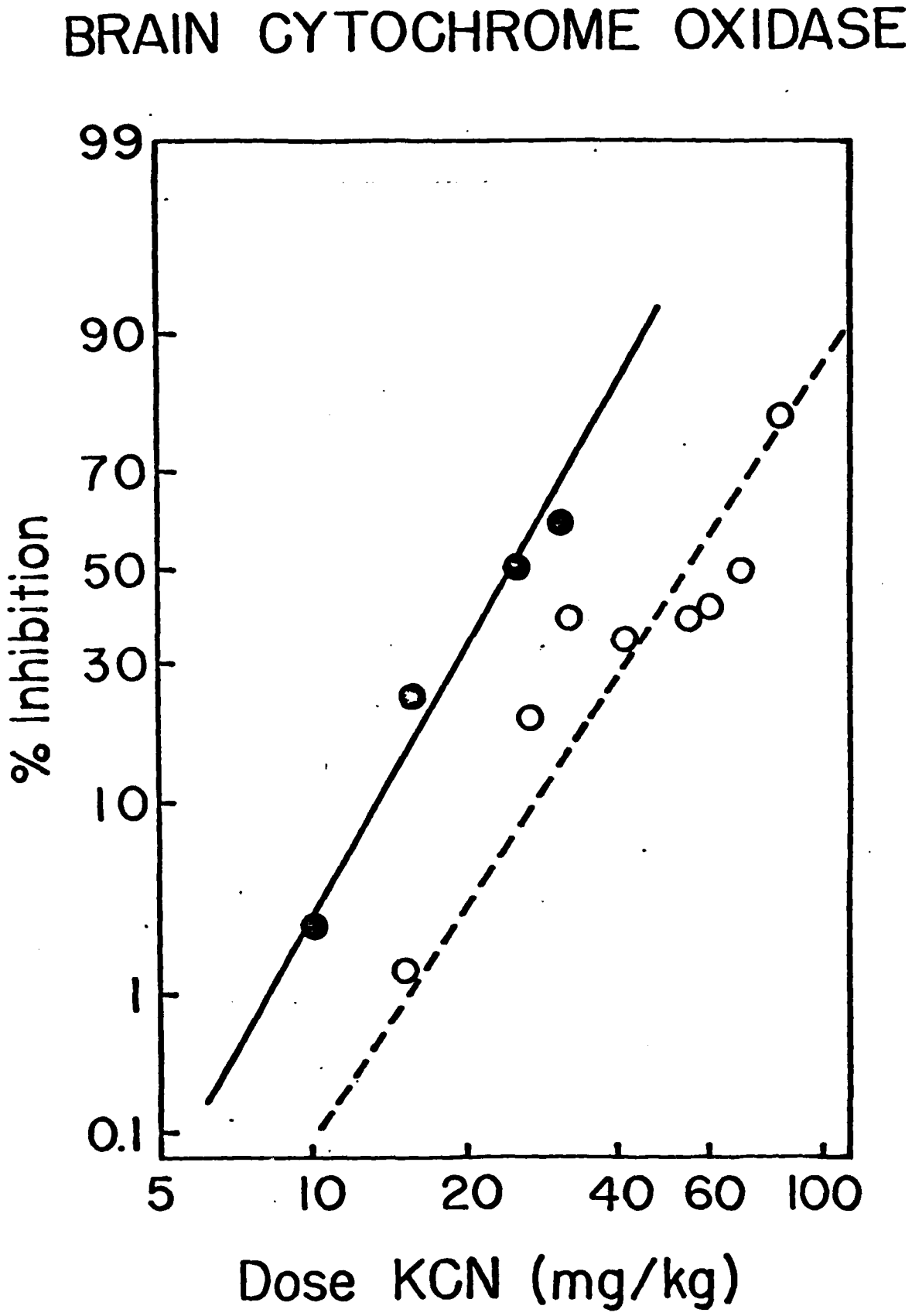

BRAIN CYTOCHROME OXIDASE
99
90
70
50
30
10
1
0.1
% Inhibition
5
10
20
40
60
100
Dose KCN (mg/kg)

Fig. 4

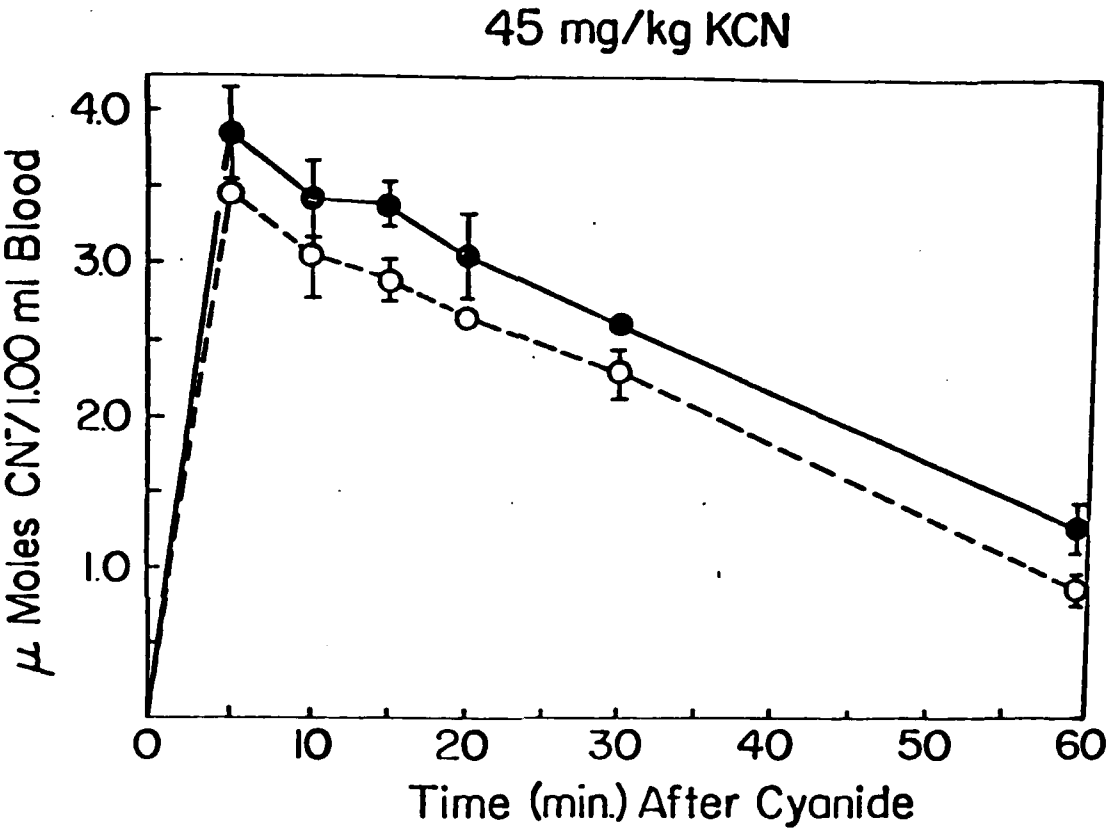

45 mg/kg KCN
μ Moles CN⁻/100 ml Blood
4.0
3.0
2.0
1.0
0
10
20
30
40
50
60
Time (min.) After Cyanide

Fig. 5

SECTION 4

Pharmacological Aspects of Cyanide and its Antagonism

PHARMACOLOGIC ASPECTS OF CYANIDE AND ITS ANTAGONISM

I. HISTORY

The early history of hydrocyanic acid and its use in toxicology and medicine has been reviewed (M.P. Earles, 1967). The earliest documented record in the use of cyanide as a poison was recorded in Germany over 200 years ago, and it was believed to be one of the more frequently employed preparations during the nineteenth century for homicides and suicides. Hydrogen cyanide probably has accounted for more known human fatalities than any other chemical known, particularly due to its application in legal executions and as a genocidal agent during World War II.

One of the most widespread potential applications of cyanide is by the military, because hydrogen cyanide has many properties of a practical chemical weapon to be employed in gas warfare (SIPRI, 1971, 1972). Also, besides the quick-kill properties, the potential mask-breaking effect is advantageous, as small molecules are not readily adsorbed on charcoal. Napoleon III was reported to apply some form of hydrogen cyanide as a tactical military weapon; however, it was not until World War I that it received widespread consideration. Approximately eight million pounds of hydrogen cyanide were manufactured by France as a chemical weapon and it was used in various mixtures called Manganite and Bincennite. These mixtures contained hydrogen cyanide and various stabilizers, dilutents, smoke markers and arsenic trichloride. The military application of hydrogen cyanide in World War I was not highly successful, partly due to the limitation of projectile size and the relative vapor density of hydrogen cyanide which limits its persistency. During World War II, various countries tested and manufactured hydrogen cyanide in larger payloads, as the vaporization of this agent caused

cooling and increased its relative vapor density. The Japanese were armed with fifty Kg bombs, and the United States employed 500 Kg bombs. It was estimated that during World War II, hydrogen cyanide was produced as a chemical weapon in Japan, U.S.S.R., and the U.S.A. in quantities from 500,000 to over 1,000,000 pounds (SIPRI, 1972). Hydrogen cyanide frequently has been discussed for clandestine use, primarily because of its quick-kill potential. Cyanide projectiles and spring-loaded devices have reportedly been employed against individuals, and canisters of hydrogen cyanide that may be shattered by an explosive charge detonated by a radio-controlled fuse have been manufactured to be employed against groups of people in buildings or vehicles. In addition to the above use of cyanide, other past applications directly related to its toxicity are as a fumigant and as a predator control agent, e.g., coyotes, by the use of solutions containing cyanide in livestock collars or spring loaded devices to spray cyanide (Sterner, 1979). Surprisingly, fatalities from accidental exposure to hydrogen cyanide are relatively infrequent, probably because it is generally recognized for its high toxicity and rapid onset action; therefore, it is handled with caution.

Hydrocyanic acid was used fairly extensively in pharmaceutical (cherry laurel) preparations as a sedative, cough suppressant, and for gastric pains until the early parts of the twentieth century. This pharmaceutical preparation derived from peach stones and almond was employed as a flavoring agent, with occasional lethal effects. In 1872, Scheele hydrogen cyanide was isolated and characterized by Scheele from prussian blue and subsequently it was isolated from this cherry-laurel preparation.

II. SOURCE

Cyanide-containing staple foods, such as cassava (B. Osuntokun, 1970) and forage plants (Schoeder, 1976), probably accounted for most

incidences of cyanide ingestion in man and range animals. However, in the past few decades the industrial production of hydrogen cyanide has been accelerating, e.g., in the U.S., production was over 700 million pounds per year by 1977 and is rapidly increasing. The predominant uses of cyanide are for the production of plastics, such as acrylonitrile, and in the metal industries. Although cyanide wastes are generated by these industries in increasing quantities, attempts have been made to control this waste cyanide; however, an appreciable amount is still discharged into the environment.

With regard to some of the industries involved, other than the plastic manufacturers, in aqueous discharge of cyanide, are the electroplating industries, as the waste streams may contain initially up to 20 percent cyanide with an estimate of approximately 20 million pounds discharged by the U.S. electroplating industry. Other industries which generate cyanide wastes in our waterways are the paint, steel and mining industries. These are relatively well known sources of contaminate; however, newer sources of cyanide contamination have evolved in the past decade. An aluminum company was found to have contaminated the underground water table from aluminum waste products near Mead, Washington. Whether this cyanide contamination in aluminum sludge is a world-wide or local problem has not been investigated. Probably the greatest future potential source of hydrogen cyanide contaminating our water supply is from coal gasification processes for making synthetic gas from coal. Many of these procedures produce high concentrations of cyanide as a by product. The cyanide concentration was of such a sufficient magnitude that procedures were attempted to recover the cyanide produced in these waste waters for economic reasons. The production of cyanide from this source would

dwarf the total present cyanide input into our environment, should this be one of the alternatives utilized to increase our energy needs. Cyanide effluent from industrial processes usually does not escape in any appreciable amounts into the environment, primarily due to proper waste management procedures which degrade the cyanide before it is discharged. The treatment techniques employed are the complete destruction of the cyanide ion or its conversion to less toxic forms. The most frequently employed procedure is alkaline chlorination, but other oxidizing agents, such as ozone and permanganate also are employed. There are other methods employed in cyanide disposal, such as electrolytic decomposition, ionizing radiation, acidification, reaction with aldehyde, ion exchange, evaporation, lagooning, and biologic degradation.

Concerning the liberation of cyanide into air, the equipping of cars with pollution control devices with malfunctioning catalytic converters, can produce varying amounts of hydrogen cyanide (Voorhoeve et al., 1975). Another source of HCN is from home fires, especially as the plastic content in the home increases the potential for the liberation of hydrogen cyanide by the combustion of various plastic materials, particularly polyurethane (Jellinek and Takada, 1977). Lastly, one of the major contributors of hydrogen cyanide in air that affects man is tobacco smoke. It should be noted that the use of low tar filter cigarettes does not necessarily decrease the hydrogen cyanide concentration of tobacco smoke.

A different source of potential HCN exposure is from the ingestion of cyanogenic foods. There are also various food products, as discussed in other chapters, which contain naturally occurring substances that can release cyanide. This is of considerable concern, as some of

these plants form the major dietary constituents in these countries. Toxicity from these cyanogenic plants is also a problem for various range animals and wildlife. The incidence of poisoning is more prevalent during drought, as these animals become less selective in their forage. Also, during these drought periods a higher concentration of cyanogenic glycosides are formed in certain plants. Besides cyanogenic plants, the fumigation of various foods with HCN can result in cyanide residues which can persist for an extensive time. There are tolerances listed for HCN residue in various food products in the United States, and these limits vary from 25 ppm in beans to 250 ppm in spices.

With increasing use of cyanide in various industrial processes, the amount of cyanide dispersed into our environment has greatly accelerated. Although it is generally recognized that cyanide has relatively low persistence in air and water under normal circumstances, it should be emphasized that when cyanide contaminates underground water, then its persistence is prolonged. In future years the potential for cyanide contamination of our underground drinking water, leading to cyanide toxicity, will be increased. Improper management of industrial cyanide effluent occasionally has resulted in fish kills and contamination of our ground water. The disposal of cyanide in waste water is still a significant problem for various industries. It is of interest to note that the U.S. Environmental Protection Agency recently filed a Refuse Act suit against the ARMCO Steel Company for discharging cyanide-containing effluents into the Houston shipping channels. This discharge was in the form of a continuous-process generated effluent stream and the case of the United States versus the ARMCO Steel Company (C.A. 70-H-1335) was prosecuted successfully by the government.

III. PHARMACODYNAMICS

A. Physiology

The physiologic effects of cyanide discussed will be confined only to those effects which are obtained with reasonable doses, with an emphasis on their effect on the intact animal. In studies when an overwhelming dose of cyanide is employed to elicit a response, as in some *in vitro* systems, these reports were not considered in this discussion, as they bear little relevance to the pharmacology and toxicology of cyanide. In concert with these studies on the effects of cyanide on cell respiration (Keilin, 1929; and Warburg, 1931), studies were conducted (Gesell and Hertzman, 1927) on the effects of cyanide on the regulation of respiration and blood flow in the intact animal. Much of the knowledge on the inhalation toxicity of hydrogen cyanide (Barcroft, 1931) and the effects of cyanide on tissue respiration in various species and organs were reported about 50 years ago (Dixon and Elliott, 1929). The responses to cyanide of the respiratory and cardiovascular systems have been studied rather intensively over the past two decades. Bradycardia in the dog from cyanide has been attributed to the activation of the chemoreceptors in the carotid body (Jacobs et al., 1971). Cyanide is also known to stimulate the peripheral chemoreceptors to promote a stimulation of ventilation. Apparently, this can occur by extracranial and carotid and aortic chemoreceptor mechanisms. However, the site(s) where these extracranial receptors are located have not been identified (Levine, 1975). The increased afferent discharges from the carotid body produced by cyanide were abolished by oxygen (von Euler et al., 1939). This phenomenon has been adapted into a clinical pulmonary function test, as oxygen abolishes the inspiratory gasp produced by cyanide in

normal individuals, whereas those who have impaired gaseous diffusion continue to exhibit this gasp (Cope and Abramowitz, 1960). One of the most consistent changes produced by cyanide is electroencephalographic (Ivanov, 1959) and electrocardiographic (Cope, 1961). Not only can these changes be produced by cyanide, but these effects can be antagonized with oxygen (Burrows et al., 1973). It should be emphasized that the mechanism involved in antagonizing the lethal effects of a compound may be quite different from those involved in the blocking of a physiologic or biochemical response induced by a sublethal dose. For example, this may explain the differences reported (Burrows et al., 1973; and Cope, 1961), on the effect of oxygen in reversing the action of cyanide on the EKG. Other cardiovascular responses to cyanide have focused on the effect of cyanide primarily to chemoreceptor reflexes and to their action on the central nervous system (Paulet, 1960; Calvelo et al., 1970; Offterdinger, 1970; and Krasney, 1971). The marked increase in arterial blood pressure could be attributed to such reflex activities, whereas the increase in central venous pressure may be due to a more direct action, producing a relative cardiac insufficiency as shown by parallel EKG alterations in rhythm.

In electroencephalographic (EEG) recordings, cyanide can produce neuroelectrical changes which correlate with alterations in cellular energy. Recordings in the frontal and parietal areas, cyanide (1-10 mg/kg) produces an abrupt loss of electrical activity followed by a prolonged period of depressed wave amplitude (Burrows et al., 1973). These periods of electrical silence, as well as EEG abnormalities, can be antagonized with oxygen and can be correlated with the role of oxygen in antagonizing cyanide lethality.

The respiratory changes produced by cyanide appear to have occurred in the same manner as the cardiovascular responses, i.e., primarily due to the result of reflex activity of chemoreceptors, as direct effect of small doses of cyanide on the activity of respiratory neurons has been reported (Brodie, 1959).

The biochemistry of cyanide with cytochrome oxidase is discussed in another chapter; however, these discussions are confined primarily to the effects of cyanide on cytochrome oxidase *in vivo*. Most of the inhibitory studies of cyanide have been *in vitro* and there have been very few studies regarding the effect of cyanide on brain cytochrome oxidase *in vivo*. The toxicity of cyanide has always been attributed to producing a decrease in the tissue utilization of oxygen or producing a "histotoxic" anoxia. Albaum et al. (1946a,b) reported that rats receiving sodium cyanide (5 mg/kg, ip) produced a 50 per cent decrease in brain cytochrome oxidase. These studies indicated that tissue anoxia induced by the inactivation of cytochrome oxidase resulted in a shift from aerobic to anaerobic metabolism and a depletion of high energy phosphorus compound, as noted by a decrease in the concentration of glycogen, phosphocreatine, and adenosine triphosphate with a concomitant increase in inorganic phosphate, lactic acid, phosphopyruvate, phosphoglycerate, and hexose diphosphates. *In vivo* studies (Schubert and Brill, 1968) indicated that rodents receiving potassium cyanide intraperitoneally produce a maximal inhibition of cytochrome oxidase within 5-10 minutes. Subsequently, Ballantyne et al. (1972) correlated the tissue concentration of cyanide with cytochrome oxidase activity and related the difference in concentration of hydrogen cyanide and KCN, with regard to tissue concentrations of cytochrome oxidase activities.

IV. PATHOLOGY

The pathology of cyanide intoxication varies according to the dose, route and duration of cyanide administration. Ballantyne (1970) conducted studies on pigs which indicated that no specific gross and histopathological differences were noted by captive bolt and intramuscular injection of hydrogen cyanide. The animals administered lethal doses of cyanide had no autopsy characteristics to specifically permit a diagnosis of "death from cyanide poisoning" to be established. It is of interest to note that no cyanide odor could be detected on opening up the body cavity and the color of the blood was variable. The cytochrome oxidase in the spinal cord was markedly inhibited; however, the cytochrome oxidase in the liver and kidneys was not significantly inhibited. This does not infer that in other cases of cyanide poisoning that the odor of "bitter almonds" may not be detected when opening the body cavities or that the blood may not be a slight pink color, but that these findings can be variable. Hurst (1940) reported in monkeys receiving potassium cyanide (im), that the most apparent lesion in the brain was in the white matter, and that necrosis was more frequent than demyelination. These findings can be produced by a single dose of potassium cyanide, but are more apt to occur on chronic administration. Experimental cyanide encephalopathy has been studied in rats (Levine and Stypulkowski, 1959; Levine, 1967; Hirano et al., 1967) by single brief exposure and the lesions were found to develop in the gray and white matter. Lesions in the white matter are more apt to occur in less severe cyanide poisonings involving particularly the corpus callosum, optic nerves, and chiasma, as well as the corpus striatum and ventral hippocampal commissure. These studies concluded that the lesions of cyanide encephalopathy were caused by the direct

effect of histotoxic anoxia, and that these lesions were not secondary to neuronal disfunction and edema.

Pathological changes also occur in the myocardium. Suzuki (1968) indicated that large doses of cyanide exert a direct effect not only on metabolism, but on the contractile components of the myocardium. The pathology in the myocardium is not surprising, as Wexler et al. (1947) indicated extensive alteration in cardiac physiology. There has been a series of studies on the tropical neuropathies produced by cassava. One of these studies, by Osuntokun (1972), demonstrated the demyelination of peripheral nerves and the decreased conduction velocity in these nerves. The implication of cassava in tropical neuropathic ataxia, with cyanide as a presumptive etiological agent, would be consistent with chronic cyanide poisoning. Although recent studies implicate that a consistent clinical, biochemical, electrophysiological, and histopathologic study in humans is observed, it is always difficult to draw an unequivocal relationship in this type of clinical field study, as other factors hitherto unknown may be a contributing cause; however, the implication of cyanide with the present evidence available is a reasonable one.

V. CYANIDE POISONING

A. Signs and Symptoms

The clinical signs and symptoms of cyanide poisoning have been described over 150 years ago by Francois Magendie and because of its widespread use, the toxic effects are well described (Polson and Tattersal, 1969). Although cyanide has been known as a rapidly acting poison, this effect, like all chemicals, is dose dependent. There are reports where cyanide may produce severe toxic effects which persist for days with the

ultimate recovery of the individual. Because cyanide has received a deserved reputation for its rapid lethal action from mass and criminal executions, as well as in suicidal and homicidal reports, it is not usually cognizant that low level chronic intoxication from cyanide does exist and can be quite incapacitating.

B. Acute and Subacute Toxicity

Acute and subacute toxicity poisoning from cyanide can vary from rather dramatic effects such as convulsions, screaming, vomiting, and bloody frothing, to occasions where death can occur rather undramatically with a slow, quiet onset of coma and subsequently death. Although many authors separate the effects of hydrogen cyanide by inhalation from the parental route, the clinical manifestations do not vary appreciably except that with inhalation the irritating effects of cyanide itself on the membranes of the eyes, oropharynx, and pulmonary tissues are more apt to be manifested. There are no distinguishing signs and symptoms which are pathonomonic for cyanide other than possibly the smell of cyanide in the breath of the victim, which has frequently been described as a burnt almond-like odor. The predominant effect which one would observe initially is a hyperpnea followed by dyspnea, and subsequently convulsions. Although cyanide is usually described as producing a "histotoxic anoxia," it is generally forgotten that in cyanide poisoning there probably is an anoxic anoxia component. The lethal effects in low lethal doses are probably directed towards cytochrome oxidase in the central nervous system, with associated cardiovascular signs at higher doses. It has been frequently described that the electrical activity of the brain may be silent when the heart is still functioning. These signs and symptoms of cyanide poisoning are described by Gettler and St.

George (1934) in as great a detail as any published reports. This report arbitrarily divides the signs and symptoms into three stages. In the dyspneic stage subsequent to the hypernea there is associated headache, vertigo, weak and rapid pulse, nausea and vomiting, and a staggering gait. There is a second stage of convulsions where the victim falls, pupils dilate, and the skin is cold, clammy, moist, and the pulse becomes more weak and rapid. There are associated opisthotonus, trismus, and urination. A third stage of asphyxia is described where heartbeat becomes irregular and slow, and there is a fall in body temperature and associated cyanosis, which is manifested in the lips, face and extremities. The victim then sinks into a coma and the frothy, bloody saliva flows from the mouth. With regard to the caustic exposure levels, the experience with cyanide has been sufficient to provide some human toxicity data. Inhalation of HCN at a concentration of 110-135 ppm (0.12-0.15 mg/l) (Fassett, 1963) has been reported to produce fatalities within a few hours, whereas HCN at a concentration of 270 ppm (0.3 mg/l) will produce immediate fatalities (Prentiss, 1937). Ingestion of a spoonful (1-5 g) of sodium or potassium cyanide usually will produce death, as the minimal lethal dose has been estimated to be approximately 0.2 g/adult (approximately 3 mg/kg). The toxicity of cyanide of course will be dependent on the rate of absorption and the ability of the body to dispose of cyanide.

C. Chronic Effects

Most of the literature on cyanide is focused on the acute effects, and the chronic effects have been almost virtually ignored. This can partly be because most of these latter reports are predomirantly clinical field reports. It is difficult to ascribe the effects specifically to

cyanide itself, because usually a mixture of chemicals is involved. The effects of long-term exposure to low concentrations of cyanide probably produces clinical signs of intoxication which are not clearly understood. In the past couple of decades there have been various correlations which implicate the effects of chronic low cyanide to specific diseases. Moreover, epidemiological studies are implicating adverse effects of cyanide to chronic exposure in occupational (El Ghawabi et al., 1975), dietary (Osuntokun, 1970) and environmental conditions. It is becoming increasingly probable that there are a series of signs and symptoms which can be ascribed to a chronic cyanide syndrome. Also, various diseases have been inferred to have a correlation with chronic low level exposure to cyanide. These would include: Nigerian nutritional neuropathy, Leber's optical atrophy, retrobulbar neuritis pernicious anemia, tobacco amblyopia, cretinism and ataxic tropical neuropathy. Future projection would suggest a greater exposure to cyanide because of newer industrial advances. The potential of chronic cyanide poisoning may be dwarfed by present developments to seek alternative energy supplies to replace petroleum. In some coal gasification procedures a considerable amount of water is employed, and this waste water contains a relatively high cyanide concentration and the mechanism of disposal of this waste water will be of considerable ecological concern.

Probably the greatest incidence of exposure to chronic low level cyanides is in the tropics, which produce various neuropathic lesions manifested in different forms. The diet of various individuals in tropical areas is predominantly cassavas, as the staple food, and this plant has a high content of cyanogenic glycosides (linamarin). The tropical neuropathies can develop into a characteristic syndrome which

is characterized by nerve deafness, optic atrophy and involvement of the sensory spinal nerve producing an ataxia (Money, 1958). In these ataxic syndromes are included such effects as nerve deafness and sensory spinal ataxia. Other signs and symptoms include stomatitis, glossitis and scrotal dermatitis. The correlation between the pathology and the clinical manifestation of this disease is relatively consistent.

In many field studies, it is difficult to assess the contribution of cyanide to these lesions due to the lack of experimental controls. However, the evidence accumulating is consistent that one of the most likely chemical toxins is cyanide, but this may be an oversimplification of a complex problem. Osuntokun and co-workers (1969) have contributed significantly to studies in this area. In some villages cassava represents approximately two-thirds of the diet, so that in addition to cyanide, there are various nutritional factors that may enter in. For example, Osuntokun and co-workers (1968) indicated that a lack of substrate for cyanide detoxification may be occurring because of a deficiency of sulfur containing amino acids in the diet. Although most studies focus on chronic cyanide intoxication (Monekosso and Wilson, 1966; Osuntokun et al., 1970), studies also indicate that deficiencies in protein and in water-soluble vitamins may, by themselves, not be important contributing factors. With regard to the clinical studies, lesions such as demyelination of peripheral nerves and decreased conduction velocity of motor nerves were elicited.

There are other neuropathies ascribing cyanide as the possible cause, such as the West Indian amblyopia, tobacco amblyopia and Leber's optic atrophy, which are characterized by a disturbance in the visual fields. One of the most convincing studies which would suggest that

chronic cyanide may be a contributing factor in these visual disturbances is that in tobacco amblyopia, administration of cyanocobalamin or hydroxocobalamin improved vision rather dramatically (Chisholm, et al., 1967). Also hydroxocobalamin was more efficacious than cyanocobalamin as one would expect (Foulds et al., 1970).

The relationship between cyanide as the etiological agent in producing human neuropathies has been investigated experimentally (Lessell, 1971; and Lessell and Kuwabara, 1974). These studies indicated that cyanide lesions produced in rats were similar to those of human disorders. However, it was pointed out that the cyanide dose necessary to produce the experimental nerve damage in rats was in the lethal range and in the rats, the corpus callosum is more sensitive to cyanide than the optic nerve. This is quite different from human disorders, as very frequently optic disturbances are the only involvement of the central nervous system.

Lastly, there is very little information on the carcinogenic, teratogenic, and mutagenic properties of cyanide, and the evidence that is available warrants additional examination. Cyanide has been employed in cancer chemotherapy in experimental animals and in man (Brown et al., 1960). There has been a longstanding hypothesis for an anticancer effect of the cyanogenic glycosides (Morrone, 1962; Krebs, 1970). These claims have been refuted by other laboratories (Greenberg, 1975; Lewis, 1977). The basis for the antitumor effect is that the cyanogenic glycoside, amygdalin (also called laetrile), is a postulated selective hydrolysis of amygdalin by a beta glucosidase, liberating cyanide, benzaldehyde at the neoplastic site. The cyanide then selectively attacks the cancer cell which was presumed to be low in rhodanese, whereas normal cells

were assumed to possess sufficient sulfur donor to detoxify the cyanide and thereby would suffer no toxic effects. Some of the opponents of this theory have indicated (Greenberg, 1975; Lewis, 1977) that many tumor tissues are not selectively enriched in beta-glucosidase nor are they low in rhodanese. Also, there are various studies indicating a lack of antitumor activity of amygdalin in model tumor cells (Hill et al., 1976; Laster and Schnabel, 1975; Levi et al., 1965; Wodinsky and Swiniaski, 1975).

VI. CYANIDE ANTIDOTES

Since cyanide poisoning has been documented in the nineteenth century, various antidotes have been proposed. It is rather a paradox that some of the cyanide antagonists proposed in the nineteenth century were quite efficacious, whereas some of the cyanide antagonists presently still recommended are ineffective. There are various reports on the development of "new" cyanide antidotes, which would be quite efficacious compared to the state of arts of other chemical poisoning; however, with regard to protection against cyanide poisoning, it would be relatively trivial because of the highly efficacious antidotal combinations presently available. Also, many of these studies become questionable when subjected to statistical analysis. Unlike most chemicals, with cyanide, it is always necessary to differentiate between the antidotal effects produced with a minimal lethal dose from those produced with massive poisoning, as the results obtained can be quite different.

A. Biotransformation of Cyanide

Subsequent to the demonstration of the existence of a thiosulfate sulfurtransferase in liver to convert cyanide to thiocyanate it became fairly apparent that this would be an effective mechanism to detoxify

cyanide (Lang, 1933a,b). Independent studies in Argentina (Hug, 1933) and the United States (Chen et al., 1933) incorporated thiosulfate into the antidotal combination to antagonize cyanide intoxication. The use of sodium thiosulfate as the sulfur donor is ideal in many aspects, as the enzyme is present in large amounts (Himwich and Saunders, 1948), has a high turnover number and this reaction is essentially irreversible (Sorbo, 1953a,b). One of the limitations of this antagonist is that the selective distribution of the agonist, hydrogen cyanide, does not parallel that of the antagonist. This enzyme is localized in the mitochondria in liver and kidneys, and the penetration of sodium thiosulfate to these sites is relatively limited. In an attempt to minimize these distribution problems, the crystalline enzyme was injected in combination with sulfur donor intravenously. This represents the first report of an enzyme being employed directly as an antidote to antagonize the toxic effects of a chemical. Various other sulfur donors have been studied (Sorbo, 1953a,b) and some of these antagonists have been employed in the treatment of cyanide poisoning (Clemedson et al., 1955).

B. Cyanide Binding

1. Methemoglobin Generating Chemicals

a. Methylene blue

Earlier studies on cyanide poisoning utilized methylene blue as a cyanide antagonist. The basis for methylene blue was related to the formation of methemoglobin, which subsequently reacted with cyanide to form cyanmethemoglobin (Wendel, 1933). Although methylene blue is still being advocated as a cyanide antagonist, it really is not an effective antidote (Chen et al., 1933) because it is a poor methemoglobin former (Bodansky, 1950). The main contribution of methylene blue to cyanide

poisoning is that it stimulated the research to uncover newer and more effective cyanide antagonists.

b. Nitrites

The first report of the efficacy of nitrites against cyanide poisoning in dogs was conducted with amyl nitrite (Pedigo, 1888). This report went relatively unnoticed, as it was published in a journal which was not widely circulated. Subsequent studies, again in dogs, employing sodium nitrite in anesthetized and unanesthetized dogs reported that sodium nitrite can protect against several lethal doses of potassium cyanide (Chen et al., 1933). Cyanide has a low affinity for hemoglobin, but has a relatively high affinity for methemoglobin. Nitrite generates methemoglobin which combines with cyanide to form cyanmethemoglobin. Methemoglobin does not have a higher affinity for cyanide than cytochrome oxidase, but there is a much larger potential source of methemoglobin than cytochrome oxidase. Therefore the efficacy of methemoglobin is primarily due to mass action, as there would be a much higher content of methemoglobin than cytochrome oxidase.

c. Cobalt compounds

In 1894 cobalt nitrate was used in the treatment of cyanide poisoning (Antal). This efficacy was confirmed (Meurice, 1900) in pigeons and rabbits; however, the use of cobalt as a cyanide antagonist did not receive widespread support predominately because it was felt that the cobalt salts were too toxic. It was over fifty years later before interest revived in using cobalt compounds in the treatment of cyanide poisoning (Mushett et al., 1952). These studies employed hydroxocobalamine in antagonizing potassium cyanide intoxication in mice. Subsequent to

these studies, various other cobalt compounds were employed and many laboratories have used these cobalt-containing compounds as cyanide antagonists, such as hydroxocobalamine or its derivatives (Mushett et al., 1952; Rose et al., 1965; Evans, 1964), cobalt gluconate or glutamate (Paulet, 1957, 1958; and Estler, 1966), cobalt histidine (Schwarzkopf and Friedberg, 1971; Mercker and Bastian, 1959), cobalt chloride (Burrows and Way, 1979; Isom and Way, 1973), and dicobalt ethylenediamine tetraacetic acid (CO_2EDTA) (Paulet, 1958). Various laboratories have reported on the successful use of cobalt EDTA in cyanide poisoning in both experimental and clinical conditions (Paulet, 1958; Mercker and Bastian, 1959; Bartelheimer et al., 1962; Nagler et al., 1978).

The rationale for the use of cobalt EDTA has a good pharmacologic basis, as it forms a stable complex with cyanide. The advocates for the use of cobalt are that the reaction is rapid, whereas the use of methemoglobin generating compounds, by its very nature, is delayed at a time where a rapid onset of action is essential.

d. Other chemicals which generate methemoglobin

Since methemoglobin formation was believed to be the pharmacologic basis for sodium nitrite, this led to the search for agents which would form methemoglobin more rapidly. Under the conditions in which amyl nitrite normally would be used, it is doubtful that it would contribute substantially to the formation of methemoglobin. Moreover, the efficacy of amyl nitrite in cyanide poisoning was raised (Jandorf and Bodansky, 1946). This has led to the search for agents which would form methemoglobin more rapidly than sodium nitrite. The first of these agents was p-aminopropiophenone, as it was shown to form methemoglobin rather rapidly (Jandorf and Bodansky, 1946). Subsequently, a series of studies by

Kiese and Weger (1969) led to the development of 4-dimethylaminophenol as the agent of choice among the aminophenol derivatives. The development of a more rapid methemoglobin former is reasonable, as Albaum et al. (1946a,b) have reported on the reactivation of cyanide inhibited cytochrome oxidase by methemoglobin in vitro studies. Moreover, various other laboratories (Paulet et al., 1960; and Weber et al., 1962) have reported on the possible limitations in the use of nitrites in cyanide intoxication may be attributed to its slow rate of formation of methemoglobin.

e. Carbonyl compounds

In addition to investigating various agents which would accelerate the formation of methemoglobin to bind cyanide, other studies were directed towards utilizing chemicals which would bind directly with cyanide. Cyanide is known to interact with various carbonyl groups to form cyanhydrin. One of these earlier reports (Cittadini et al., 1971 and 1972) indicated that sodium pyruvate can reverse the inhibitory effects of cyanide on tumor cells, and subsequently reported its efficacy in antagonizing the lethal effects of cyanide. The minimal protective effect of sodium pyruvate alone was subsequently confirmed by Schwartz et al. (1979), and these studies were further extended in mice. Although sodium pyruvate is not as efficacious as sodium nitrite and does not appear to enhance the effect of sodium nitrite, it does potentiate the antidotal effect of sodium thiosulfate. However, the sodium pyruvate-sodium thiosulfate combination is not as effective as the classic sodium nitrite and sodium thiosulfate combination, but the addition of sodium pyruvate to the sodium nitrite-sodium thiosulfate combination further enhances the antidotal effect. The use of sodium pyruvate as a cyanide antagonist has theoretical advantages, as it would not be dependent on

forming a second compound for activity. Also, pyruvate is transported by an active mechanism; therefore, pyruvate has the potential of being a superior cyanide antagonist than methemoglobin former, as it can distribute to sites of cyanide localization. Lastly, as a possible supplement to the nitrite-thiosulfate combination, it may provide a basis to decrease the dose of sodium nitrite, as this latter antagonist has caused fatalities.

2. Miscellaneous Antagonists

a. Oxygen

Since cyanide is believed to exert its toxic effects by the inhibition of cytochrome oxidase (Keilin, 1930), then oxygen transport and oxygen tension are usually adequate, and only the cellular utilization of oxygen is depressed. Therefore, the administration of oxygen should serve no useful purpose in antagonizing cyanide intoxication; yet, it was reported that oxygen is useful as an integral part of the treatment (Way et al., 1966 and 1972; Way and Sheehy, 1968); rather than as an adjunct (Cope, 1961; Gordh and Norberg, 1947). Oxygen potentiation was demonstrated not only prophylactically but therapeutically (Sheehy and Way, 1968). The most striking potentiation observed is when oxygen is administered in combination with sodium nitrite and sodium thiosulfate and the mechanism for this potentiation is still not apparent.

VII. TREATMENT OF CYANIDE POISONING

The treatment of cyanide poisoning at the present state of the art can best be summarized by: 1) removal from the site of contamination; 2) supportive treatment; 3) antidotal combinations which will not only bind but also detoxify cyanide.

The treatment of cyanide poisoning, in contrast to most chemical poisoning, can be treated by a variety of efficacious agents. Under

such circumstances, a variety of recommendations have been proposed for the treatment of cyanide poisoning and there is no unanimity with regard to the treatment of cyanide poisoning. This is not surprising, as different experimental designs and species of animal were employed to test the efficacy of the proposed antidote. Moreover, cyanide poisoning occurs with sufficient frequency that there have been various reports with regard to the "successful" treatment of cyanide intoxication with a variety of methods.

Although the mechanisms of action of cyanide in inhibiting enzymes are discussed in other chapters, it is necessary to relate this to the mechanism of cyanide intoxication. The action of cyanide with cytochrome oxidase is rather complex and this brief discussion is an oversimplification as it pertains only to cyanide antagonism. Cytochrome oxidase is the terminal oxidative respiratory enzyme in the mitochondria, and it is important to catalyze the tissue utilization of oxygen. Therefore, with the inhibition of this enzyme by cyanide, a histotoxic anoxia occurs as aerobic metabolism is inhibited. The organism attempts to compensate by shifting to anaerobic metabolism, with the accumulation of such products as lactate, pyruvate, glucose, etc. Therefore, in the underlying pathologic mechanism, the tissues have sufficient oxygen but are unable to utilize the oxygen in tissue metabolism. The brain is probably the most sensitive organ to cyanide and therefore most of the attention has focused on brain cytochrome oxidase. It should be emphasized that other organs, such as the heart, can be affected. The primary focus on cytochrome oxidase is attributed to the sensitivity of this enzyme to cyanide. Secondly, this enzyme is located rather critically in the biochemical metabolic chain.

AD-A115 669 WASHINGTON STATE UNIV PULLMAN DEPT OF PHARMACOLOGY F/G 6/15
CYANIDE - MECHANISM OF PROPHYLAXIS AND EFFECT ON CYTOCHROME OXI--ETC(U)
AUG 81 J L WAY DAMD17-80-C-0134
UNCLASSIFIED NL

2 OF 2

END
DATE FILMED
7-82
DTIC

The discussion on the treatment of cyanide poisoning can be separated into three arbitrary areas.

A. The General Supportive Treatment

Since there are various effective specific antidotes for the treatment of cyanide poisoning, the general supportive treatment frequently is overlooked. Because of the rapidity of action of cyanide, there are very few clinical studies with regard to acute cyanide poisoning. As indicated earlier, although various reports and textbooks describe in detail the signs and symptoms of cyanide poisoning, the fact remains that in the absence of a suitable history, the diagnosis of cyanide poisoning is difficult. Although this was a case report of a single incident of a man ingesting potassium cyanide, the only treatment employed, were supportive, as a diagnosis of cyanide poisoning was not established (Graham et al., 1977). Most of the supportive treatment was directed towards the lactic acid-dosis and pulmonary edema. These studies represent a clinically well-documented study on cyanide poisoning in man. The dose of cyanide was established at 600 milligrams, and the blood level of cyanide was reportedly 2.0 microgram/milliliter 12 hours after admission to the hospital. The predominant importance of these studies (Graham et al., 1977) is to reemphasize that even though effective antidotes are available, the general supportive treatment of any poisoning should not be ignored. It may be lifesaving, particularly when the diagnosis of cyanide poisoning has not been established.

B. Nitrite-Thiosulfate-Oxygen

Various combinations of proposed cyanide antagonists were reported in animals receiving cyanide with varying efficacy. The reports on the synergistic protective effect of the nitrites with sodium thiosulfate

were reported independently by investigators in Argentina and the United States (Hug, 1933; and Chen et al., 1933). Each of these drugs have been known, for quite some time, to antagonize cyanide intoxication; however, these were the first trials of this antidotal combination and it is still one of the most efficacious antidotes against cyanide poisoning. This is partly because the antidotal combination was based on a rational pharmacologic basis of a dual mechanism of action involving the binding of cyanide as well as its biotransformation. The nitrites were employed to oxidize hemoglobin to methemoglobin so that cyanide could combine with methemoglobin to form cyanmethemoglobin. This was based on the early studies (Bernard, 1932) which indicated that cyanmethemoglobin was much less toxic than free cyanide, and that cyanide has a much higher affinity for methemoglobin than hemoglobin.

The proprietary mixture of sodium thiosulfate and sodium nitrite which are employed in cyanide poisoning, particularly in veterinary practices, contain too low a dose to obtain maximal antidotal efficiency. Nevertheless, these proprietary mixtures can protect against 6 LD-50 doses of cyanide. However, with the adjustment of the dose of sodium nitrite and sodium thiosulfate on a reasonable basis, it was possible to raise the protection over that of the proprietary mixture by almost threefold. These studies were conducted in sheep, as they are one of the animals which are more apt to be poisoned by cyanide (Burrows and Way, 1979). The nitrite-thiosulfate combination was raised so that the amount of methemoglobin formed would be approximately 40 percent. The sodium thiosulfate was increased so that it more closely paralleled results which could be obtained under experimental conditions. It should be pointed out that the cyanide was administered orally, and a five minute

waiting period was instituted prior to initiation of the antidote so that the toxic effects of cyanide poisoning could be fully elicited. The use of sodium nitrite and sodium thiosulfate are not without their toxic effects. As it has been pointed out by Berlin (1970), care must be taken in the administration of the sodium nitrite and sodium thiosulfate combination, particularly in children. It was suggested that the standard treatment in children should be altered to avoid the potential lethal methemoglobin formation. It is recommended that children under 25 kilogram should have the dose of sodium nitrite adjusted, as if the adult dose is employed, it can be potentially lethal for children. The use of sodium nitrite and sodium thiosulfate as an antidotal combination in the treatment of cyanide intoxication and the clinical cases of poisoning were summarized for humans (Chen and Rose, 1952) and in veterinary practice in sheep and cattle (Clawson et al., 1934).

With regard to the use of oxygen with sodium nitrite and sodium thiosulfate, since there appears to be no hazard in using oxygen in this manner, and the procedure could be lifesaving, its adoption as a routine measure appears justified.

C. Cobalt

There are various proponents for the use of cobalt EDTA in the treatment of cyanide poisoning (Paulet, 1960). It is of interest to note that although cobalt salts were among the earliest antidotes employed to antagonize the lethal effects of cyanide (Antal, 1894), they did not receive widespread acceptance, because of their toxicity, until hydroxocobalamine and cobalt EDTA were introduced. Whether these compounds have appreciably lower toxicity on a molar basis with regard to cobalt is one of the questions still raised. Recent studies (Nagler

et al., 1978; Hilman et al., 1974; Naughton, 1974) appear to relate The ventricular arrhythmias primarily to cobalt EDTA itself. Strangely enough, many studies on cobalt compounds suggest that cobalt should be employed with sodium thiosulfate, as in most cases the thiosulfate enhances the antidotal effects of cobalt (Friedberg, 1968; Schwarzkopf and Friedberg, 1971; Isom and Way, 1973). However, cobalt EDTA is usually employed alone and the proprietary mixture contains no sulfur donor. Various investigators have suggested that cobalt EDTA should be employed as one of the cyanide antagonists, and infer that a cobalt chelate or an aminophenol derivative in combination with sodium thiosulfate might replace the classic antidotal nitrite-thiosulfate combination. Objections to the nitrite-thiosulfate combination have been attributed to their slow onset of action and slow detoxifying capacity (Friedberg, 1968). These conclusions were based on the rate of methemoglobin formation (Kiese and Weger, 1969) and measurement of the physiological effects of cyanide and/or rate of cyanide excretion. These conclusions (Burrows and Way, 1973) indicated that some of these measurements may not necessarily be reliable parameters in assessing the therapeutic efficacy of the antidotal potential against the lethal effects of cyanide. It is of interest to note that there is a species specificity with regard to the susceptibility to cobalt. Whereas cobalt-thiosulfate combinations were much more efficacious than the nitrite-thiosulfate in mice (Isom and Way, 1972), in sheep the tolerance to cobalt salts was much lower. Since cobalt is believed to exert its effect primarily by combining directly with cyanide ion, it is dependent on a molar ratio between cobalt to cyanide. When the dose of cobalt is adjusted to a level which can be tolerated in the sheep, then the cobalt-thiosulfate combination was not as efficacious as the nitrite-

thiosulfate in mice or sheep (Burrows and Way, 1977 and 1979). These studies are primarily to indicate that in investigations conducted by various laboratories, there are differences in experimental conditions, animal species and dose of antagonist employed which makes the comparison of relative efficacy more difficult.

Unlike the addition of oxygen to the nitrite-thiosulfate antidotal combination, there may be a much greater hazard in the use of cobalt compounds. Even though it could be lifesaving, a careful study of the toxicity of cobalt is essential, since its use under clinical conditions are manifesting signs of cobalt toxicity, particularly with reference to ventricular arrhythmias.

VIII. ASSESSMENT OF TREATMENT OF CYANIDE INTOXICATION

In most chemical poisoning there is very little that can be done, other than supportive treatment or the use of antagonists with only a minimal degree of efficacy. However, the treatment of cyanide intoxication has been blessed with a variety of agents which are quite efficacious, and for this reason there have been differences of opinion with regard to how these intoxications should be treated. The classic treatment of cyanide poisoning was developed almost fifty years ago (Chen et al., 1933; Hug, 1933). This antidotal combination was developed on a rational pharmacologic basis, and it is one of the antidotal combinations still employed in the treatment of cyanide poisoning. The only improvement to this antidotal combination is the use of oxygen (Way et al., 1966; Sheehy and Way, 1968), as it potentiates the effectiveness of the nitrite-thiosulfate combination. There are some concerns raised with regard to the use of this antidotal combination and this is partly attributed to the toxicity of nitrite (Berlin, 1970). Also some questions have been

raised with regard to its onset of action, particularly sodium nitrite (Friedberg, 1968; Kiese and Weger, 1969). Various agents have been developed which have a more rapid rate of methemoglobin formation and there are various proponents for replacing sodium nitrite with 4-dimethylaminophenol (Kiese and Weger, 1969). With regard to the use of a cobalt-containing compound, to also bind cyanide its proponents have focused on the use predominantly on cobalt EDTA (Paulet, 1960). The use of cobalt EDTA is widespread and their proponents focus on its rapid direct action to form a stable cyanide complex, whereas most other compounds are dependent on the indirect generation of methemoglobin. However, there are some concerns raised with regard to the toxicity of this cobalt compound.

BIBLIOGRAPHY

Albaum, H.G., Tepperman, J., and Bodansky, O., (1946a) J. Biol. Chem. 163:641-647.

Albaum, H.G., Tepperman, J., and Bodansky, O., (1946b) J. Biol. Chem. 164:45-51.

Antal, J., (1894) Arch. Med. 3:117-128.

Ballantyne, B., (1970) Medicine, Science and the Law, Sweet and Maxwell Ltd., London, UK, pp. 171-178.

Ballantyne, B., Boardman, S.P., Bright, J., Coffee, D.J., Webber, T.D., and Williams, P., (1972) Br. J. Pharmacol. 44:382-383.

Barcroft, J., (1931) J. of Hygiene 31:1-34.

Bartelheimer, E.W., Friedberg, K.D., and Lendle, L., (1962) Archs. int. Pharmacodyn. Ther. 139:99-108.

Berlin, C.M., (1970) Pediatrics 46:193-6.

Bodansky, O., (1951) Pharmacol. Rev. 3:144-196.

Brodie, D.A., (1959) J. Pharmacol. Exp. Therap. 126:264-269.

Brown, W.E., Wood, C.D., and Smith, A.N., (1960) Am. J. Obstet. Gynecol. 80: 907-918.

Burrows, G.E., Liu, D.H.W., and Way, J.L., (1973) J. Pharmacol. Exp. Ther. 184:739-748.

Burrows, G.E. and Way, J.L., (1977) Amer. J. Vet. Res. 38:223-227.

Burrows, G.E. and Way, J.L., (1979) Amer. J. Vet. Res. 40:613-617.

Calvelo, M.G., Abboud, F.M., Ballard, D.R., and Abdel-Sayed, W., (1970) Circ. Res. 27:259-276.

Chen, K.K., Rose, C.L., and Clowes, G.H.A., (1933) Proc. Soc. Exp. Biol. Med. 31:250-252.

Chen, K.K. and Rose, C.L., (1952) J. Am. Med. Ass. 149:113-119.

Chisholm, I.A., Bronte-Stewart, J., and Foulds, W.S., (1967) Lancet (Great Britain) 2:450-451.

Cittadini, A., Galeotti, T., and Terranova, T., (1971) Experientia 27: 633-635.

Cittadini, A., Caprino, L., and Terranova, T., (1972) Experientia 28:943-944.

Clawson, A.B., Bunyea, H., and Couch, J.F., (1934) J. Wash. Acad. Sci. 24:369-385.

Clemedson, C., Hulfman, H.I., and Sorbo, B., (1955) Acta Phys. Scand. 35:31-35.

Cope, C. and Abramowitz, S., (1960) Am. Rev. Resp. Dis. 81:321-328.

Cope, C., (1961) J. Am. Med. Ass. 175:1061-1064.

Dixon, M. and Elliott, K.A.C., (1929) Biochem. J. 23:812-830.

Earles, M.P., (1967) Med. Hist. 11:305-313.

El Ghawabi, S.H., Gaafar, M.A., El-Saharti, A.A., Ahmed, S.H., Malash, K.K., and Fanes, R., (1975) Br. J. Ind. Med. 32:215-219.

Estler, C.J., (1966) Arch. exp. Path. Pharmak. 252:305-313.

Evans, C.L., (1964) Brit. J. Pharmac. Chemother. 23:455-475.

Fassett, D.W., (1963) Industrial Hygiene and Toxicology, Vol. 2, 2nd ed., D.W. Fassett and D.D. Irish, Eds., John Wiley and Sons, Inc., New York, pp. 1991-2036.

Foulds, W.S., Cant, J.S., Chisholm, I.A., Bronte-Stewart, J., and Wilson, J., (1968) Lancet (Great Britain) 1:896-897.

Friedberg, K.D., (1968) Arch. Toxikol. 24:41-48.

Gesell, R. and Hertzman, A.B., (1927) Am. J. Physiol. 83:420-444.

Gettler, A.O. and St. George, A.V., (1934) J. Clin. Pathol. 4:429-437.

Gordh, T. and Norberg, B., (1947) Acta Physiol. Scand. 13:26-34.

Graham, D.L., Lamar, D., Theodore, J., and Robin, E.D., (1977) Arch. Int. Med. 13:1051-1055.

Greenberg, D.M., (1975) West. J. Med. 122:345-348.

Hill, G.J., Shine, T.E., Hill, H.Z., and Miller, C., (1976) AKR Leukemia Cancer Res. 36:2102-2107.

Hilmann, B., Bardham, K.D., and Bain, J.T.B., (1974) Postgrad. Med. J. 50:171-174.

Himwich, W.A. and Saunders, J.P., (1948) Am. J. Physiol. 153:348-354.

Hirano, A., Levine, S., and Zimmerman, H.M., (1967) J. Neuropathol. Exp. Neurol. 26:200-213.

Hog, E., (1933) Revta. Soc. Argent. de Biol. 9:91-97.

Isom, G. and Way, J.L., (1972) Toxicol. and Appl. Pharmacol. 24:449-456.

Isom, G.E., Liu, D.H.W., and Way, J.L., (1975) Biochem. Pharmacol. 24:871-875.

Ivanov, J.P., (1959) Pharmacol. and Toxicol. (USSR) 22:476-479.

Jacobs, L., Sampson, S.R., and Comroe, J.H., (1971) Am. J. Phys. 220:472-476.

Jandorf, B.J. and Bodansky, O., (1946) J. Ind. Hyg. a Toxicology 28:125-132.

Jellinek, H.H.G. and Takada, K., (1977) J. Polymer Sci. 15:2269-2288.

Keilin, D., (1929) Proc. Roy. Soc. B 104:206-252 (1929).

Kiese, M. and Weger, N., (1969) Europ. J. Pharmacol. 7:97-105.

Krasney, J.A., (1971) Amer. J. Physiol. 220:1361-1366.

Krebs, E.T., Jr., (1970) J. Appl. Nutr. 22:75-86.

Lang, K., (1933a) Biochem. Z. (West Germany) 259:243-256.

Lang, K., (1933b) Biochem. Z. (West Germany) 263:262-267.

Laster, W.R. and Schnabel, F.M., (1975) Cancer Chemothe. Rep. Part 1 50:951-965.

Lessell, S., (1971) Arch. Ophthalmol. 86:194-204.

Lessell, S. and Kuwabara, T., (1974) Invest. Ophthalmol. 13:748-756.

Levi, L., French, W.D., Bickis, I.J., and Henderson, I.W.D., (1965) Can. Med. Assoc. J. 92:1057-1061.

Levine, S. and Stypulkowski, W., (1959) Am. Med. Ass. Arch. Path. 67:306-323.

Levine, S., (1967) J. Neuropath. Exp. Neurol. 26:214-222.

Levine, S., (1975) J. Appl. Phys. 39:199-204.

Lewis, J.P., (1977) West. J. Med. 127:55-62.

Mercker, H. and Bastian, G., (1959) Arch exp. Path. Pharmakol. 236:449-458.

Meurice, J., (1900) Arch. int. Pharmacodyn. 7:12-53.

Monekosso, G.L. and Wilson, J., (1966) Lancet (Great Britain) 1:1062-1064.

Money, G.L., (1958) W. Afr. Med. J. (London) 7:58-62.

Morrone, J.A., (1962) Exp. Med. Surg. 20:299-308.

Mushett, C.W., Kelley, K.L., Boxer, G.E., and Rickards, J.C., (1952) Proc. Soc. Exp. Med. 81:234-237.

Nagler, J., Provoust, R.A., and Parizel, G., (1978) J. Occ. Med. 20:414-416.

Naughton, M., (1974) Anaesth. Intens. Care 4:351-356.

Offterdinger, H., (1970) Naunyn-Schmiedberg Archiv. Pharmakol. 266:416.

Osuntokun, B.O., Durowoju, J.E., McFarlane, H., and Wilson, J., (1968) Br. Med. J. (Great Britain) 3:647-649.

Osuntokun, B.O., Monekosso, G.L., and Wilson, J., (1969) Br. Med. J. (Great Britain) 1:547-550.

Osuntokun, B.O., (1970) Br. J. Nutr. 24:797-800.

Osuntokun, B.O., Aladetoyinbo, A., and Adeuja, A.O.G., (1970) Lancet (Great Britain) 2:372-373.

Osuntokun, B.O., (1972) Plant Foods Hum. Nutr. (Great Britain) 2:215-266.

Paulet, G., (1957) Presse Med. 65:573-576.

Paulet, G., (1958) J. Physiol. (Paris) 50:438-442.

Paulet, G., (1960) L'intoxication cyanhydrique et son treatment, Masson and Co., Paris, pp. 48-51.

Pedigo, L.G., (1888) Tr. M. Soc. Virg. 19:124-131.

Polson, C.J. and Tattersall, R.N., eds., (1969) Hydrocyanic Acid and Cyanides, Clinical Toxicology, 2nd ed., J.B. Lippincott Co., Philadelphia, PA, pp. 128-159.

Prentiss, A.M., (1937) Systemic Toxic Agents, Chemicals in War, McGraw-Hill Book Co., Inc., New York, pp. 170-176.

Rose, C.L., Worth, R.M., Kikuchi, K., and Chen, K.K., (1965) Proc. Soc. Exp. Biol. Med. 120:780-783.

Schroder, U.N., (1976) Proc. Soil and Crop Sci. Soc., Florida 36:195-197.

Schubert, J. and Brill, W.A., (1968) J. Pharmacol. 162:352-359.

Schwartz, C., Morgan, R.L., Way, L.M., and Way, J.L., (1979) Toxicol. Appl. Pharmacol. 50:437-441.

Schwarzkopf, H.A. and Friedberg, K.D., (1971) Arch. Toxicol. 27:111-123.

Sheehy, M. and Way, J.L., (1968) J. Pharmacol. 161:163-168.

SIPRI (Stockholm Int. Peace Res. Inst.), (1971) Problems of Chemical and Biological Warfare, I. The Rise of CB Weapons, Humanities Press Inc., NJ.

SIPRI, (1972) Problems of Chemical and Biological Warfare, II. CB Weapons Today, Humanities Press Inc., NJ.

Sörbo, B.O., (1953a) Acta. Chem. Scand. 7:1129-1136.

Sörbo, B.O., (1953b) Acta. Chem. Scand. 7:1137-1145.

Sterner, R.T., (1979) Bull. Env. Contam. Toxicol. 23:211-217.

Suzuki, T., (1968) Tohoku J. Exp. Med. 95:271-287.

Von Euler, U.S., Liljestrand, G., and Zotterman, Y., (1939) Scand. Arch. Physiol. 83:132-152.

Voorhoeve, R.J.H., Patel, C.K.W., Trimble, L.E., and Kerl, R.J., (1975) Science 190:149-151.

Warburg, O., (1931) Biochem. Z. 231:493-497.

Way, J.L., Gibbon, S.L., and Sheehy, M., (1966) J. Pharmacol. Exp. Ther. 153:381-385.

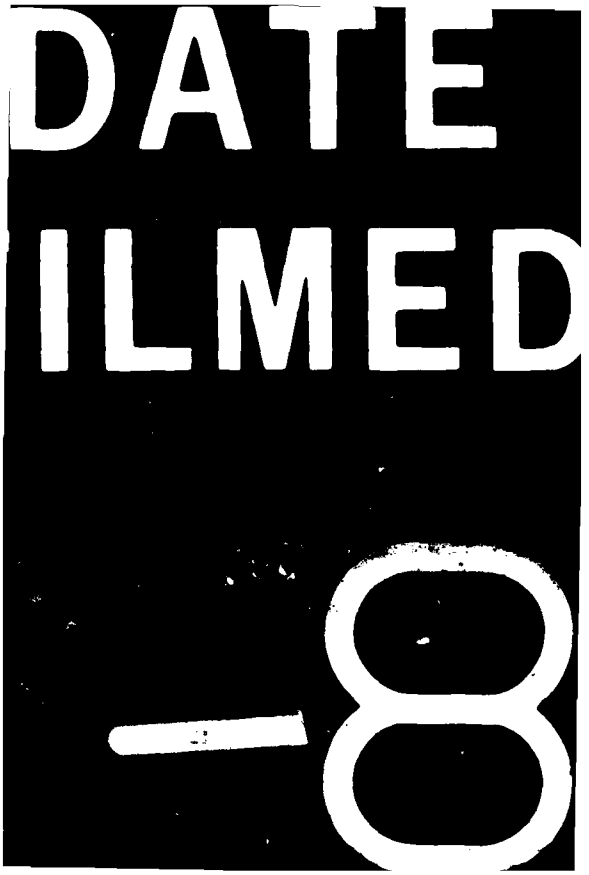
Way, J.L., End, E., Sheehy, M.H., Miranda, M., Feitknecht, U.F., Burrows, G.E., Gibbon, S.L., and Bachand, R., (1972) Toxicol. and Appl. Pharmacol. 22:415-421.

Weber, D., Friedber, K.D., and Lendle, L., (1962) Arch. exp. Path. u. Pharmak. 244:1-16.

Wendel, W.B., (1933) J. Am. Med. Ass. 100:1054-1055.

Wexler, J., Whittenberger, J.L., and Dumke, P.R., (1947) Am. Heart J. 34:163-173.

Wodnisky, I., and Swiniarski, J.K., (1975) Cancer Chemother. Rep. Part 1 59:939-950.


DATE
ILMED
-8